Monitoring of phosphatase and kinase activity using ^{31}P NMR

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Supporting Information

Contents

1 Measurement deviation

Table S1. Differentiations between theoretical and experimental concentrations of phosphorus-containing substances. Experimental concentrations were calculated with ^{31}P NMR calibration curves.

	cAMP		AMP		glucose-6-phosphate	
Theo. (mM)	2	5	2	5	2	5
Exp. (mM)	1.96	4.73	1.91	4.89	1.92	5.46
△diff.	2.0%	5.4%	4.5%	2.2%	4.0%	9.2%

Δ diff. = |Theo. − Exp.| Theo. $^{-1}$. *100%. All of the differences are less than 10%.

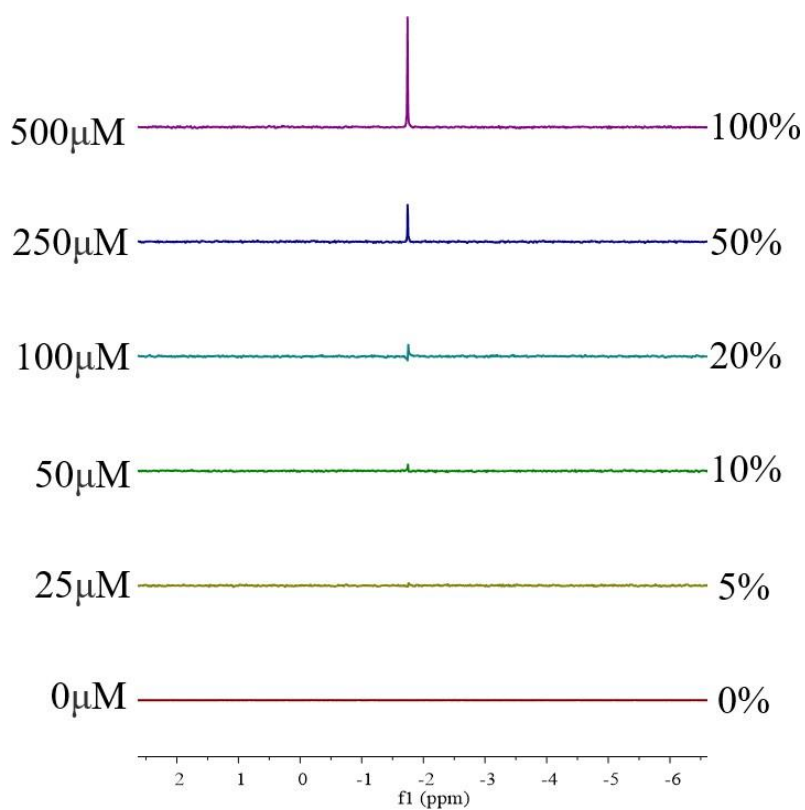## 2 ³¹P NMR spectra of cAMP

2 ³¹P NMR spectra of cAMP

Fig. S1. ³¹P NMR spectra of different concentrations of cAMP to quantify the accuracy of cAMP/AMP concentration measurements. 25μM, 50μM, 60μM, 100μM, 125μM, 250μM, and 500μM cAMP were dissolved in 10% D_2O, and 500μM cAMP was converted into 100%, it can be seen from the figure that the peak intensity of cAMP changes with the concentration of cAMP There is a positive correlation, the higher the concentration of cAMP, the higher the peak, and vice versa.
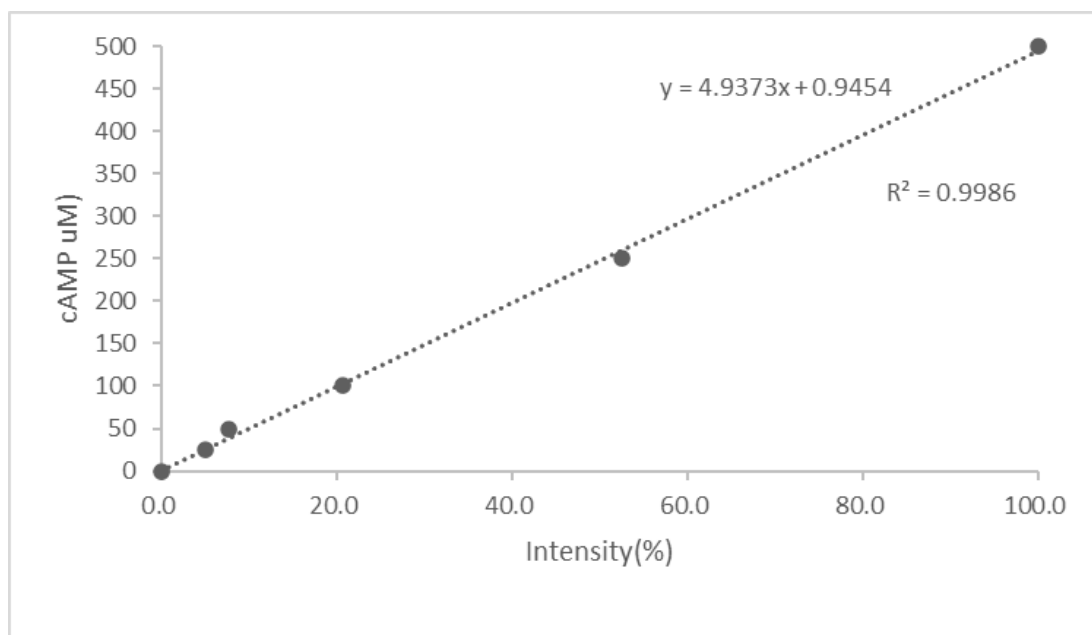
Fig. S2. Calibration curves for different concentrations of cAMP. The peak area of the ^{31}P NMR spectrum of cAMP with different concentrations in the above figure is the original data, and the cAMP peak area of 500 μM is converted into 100% to obtain the linear relationship between the cAMP concentration and the peak area, R^2=0.9986 indicates the peak area and the concentration. Linear correlation is good. And another two concentrations were used to verify the standard curve, and the error between the experimental value and the real value was within 10%. From the above experimental results, it can be known that the concentration of cAMP can be quickly and accurately obtained by ^{31}P NMR technology.
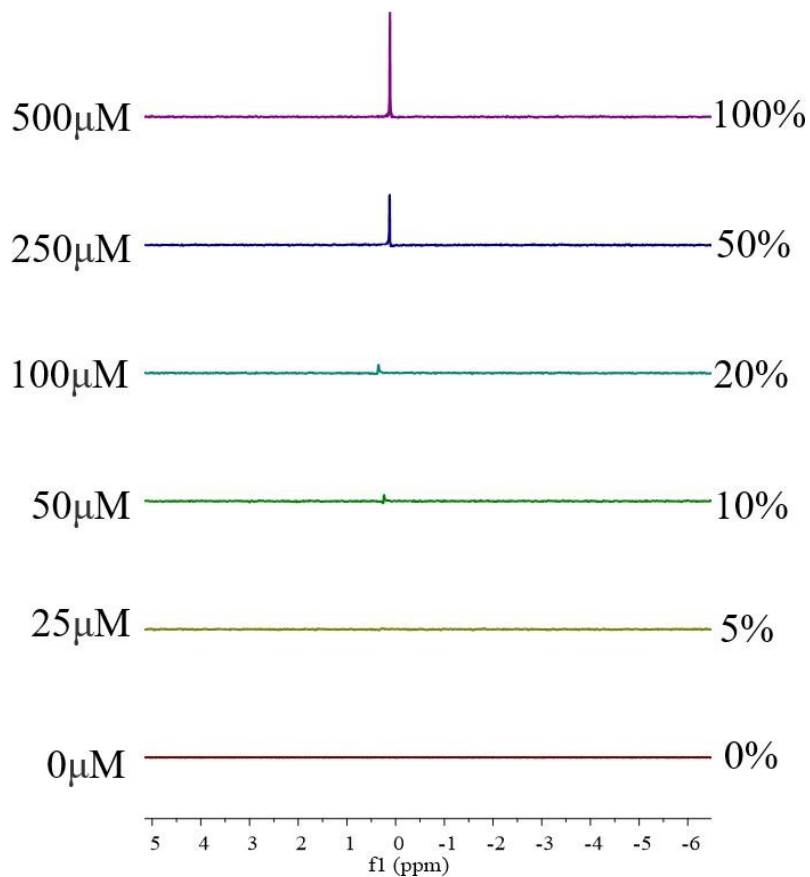
3 ³¹P NMR spectra of AMP

Fig. S3. ³¹P NMR spectra of different concentrations of AMP to quantify the accuracy of cAMP/AMP concentration measurements. 25μM, 50μM, 60μM, 100μM, 125μM, 250μM, and 500μM cAMP were dissolved in 10% D_2O, and 500μM AMP was converted into 100%, it can be seen from the figure that the peak intensity of AMP changes with the concentration of AMP There is a positive correlation, the higher the concentration of AMP, the higher the peak, and vice versa.
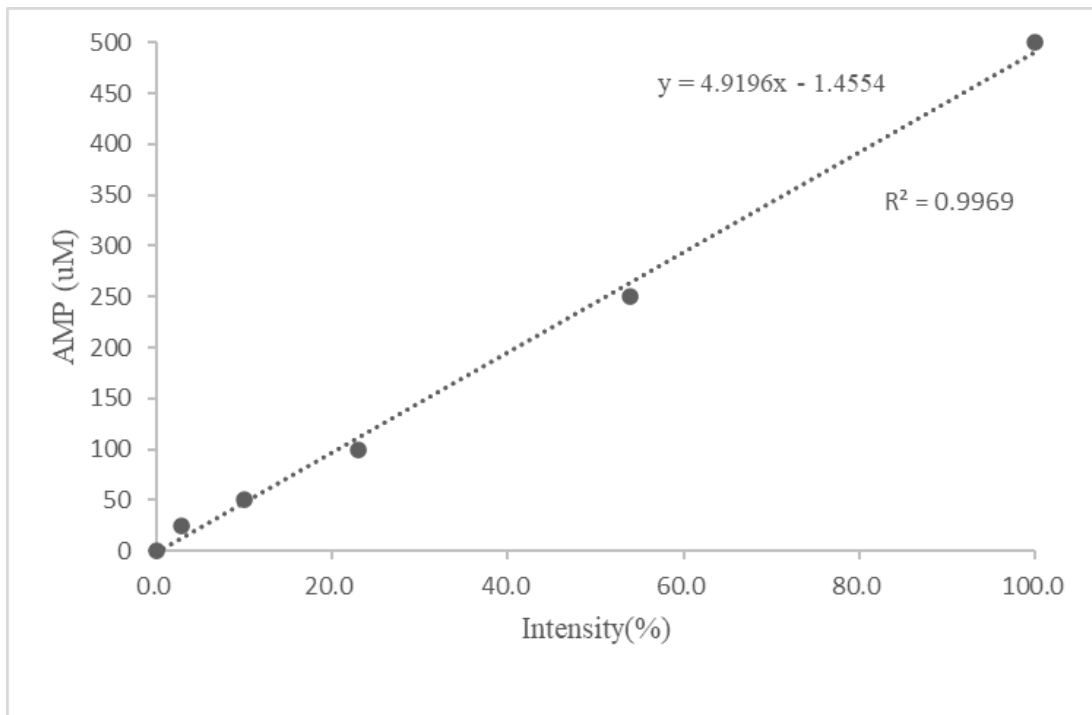
Fig. S4. Calibration curves for different concentrations of AMP. The peak area of the ^{31}P NMR spectrum of AMP with different concentrations in the above figure is the original data, and the AMP peak area of 500 μM is converted into 100% to obtain a linear relationship between cAMP concentration and peak area. R^2=0.9969 indicates that the peak area and concentration Linear correlation is good. And another two concentrations were used to verify the standard curve, and the error between the experimental value and the real value was within 10%. From the above experimental results, it can be known that the concentration of AMP can be quickly and accurately obtained by ^{31}P NMR technology.

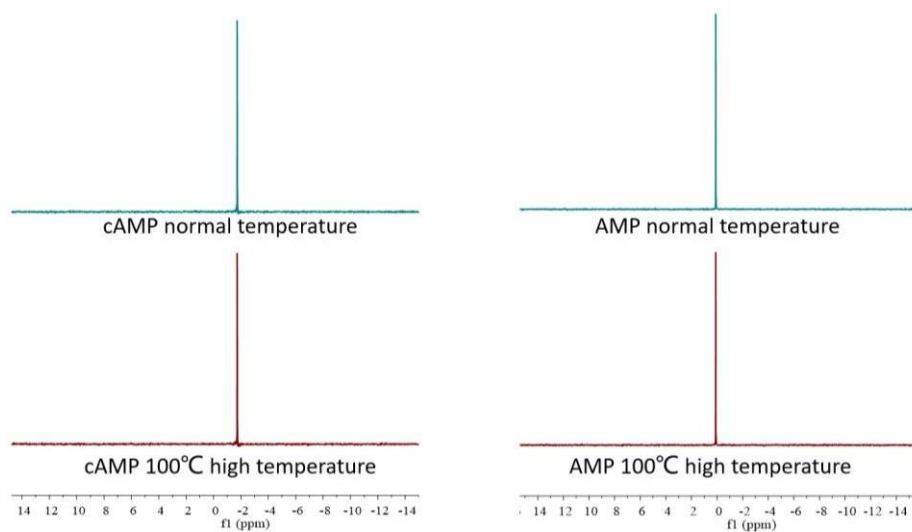## 4 Thermal stability of cAMP and AMP

Fig. S5. Thermostability experiments of cAMP and AMP. Since the enzyme needs to be inactivated in this experiment, and high temperature inactivation is used, the thermal stability of the reactants needs to be tested. The cAMP/AMP samples of equal concentration are divided into two parts, and one part is subjected to 5min 100°C inactivation. High temperature treatment, no treatment at room temperature, and ^{31}P NMR detection found that the peak areas of the samples treated at room temperature and high temperature were unchanged, so short-term high temperature treatment did not affect cAMP/AMP.

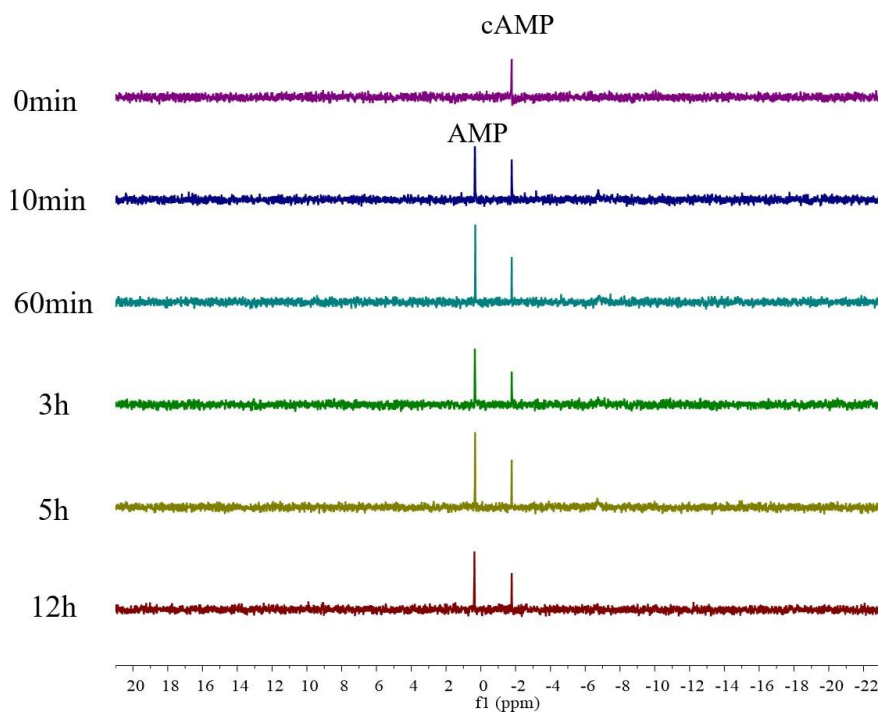## 5 PDE II enzyme activity time gradient assay

Fig. S6. Enzymatic activity time gradient assay for PDE II. We have analyzed the enzymatic activity of PDE II in real-time by hydrolysis of cAMP or AMP production. Under our experimental conditions, a reaction time of 10 minutes was sufficient to produce detectable amounts of AMP and the amount of substrate or product remained essentially unchanged after 10 minutes.

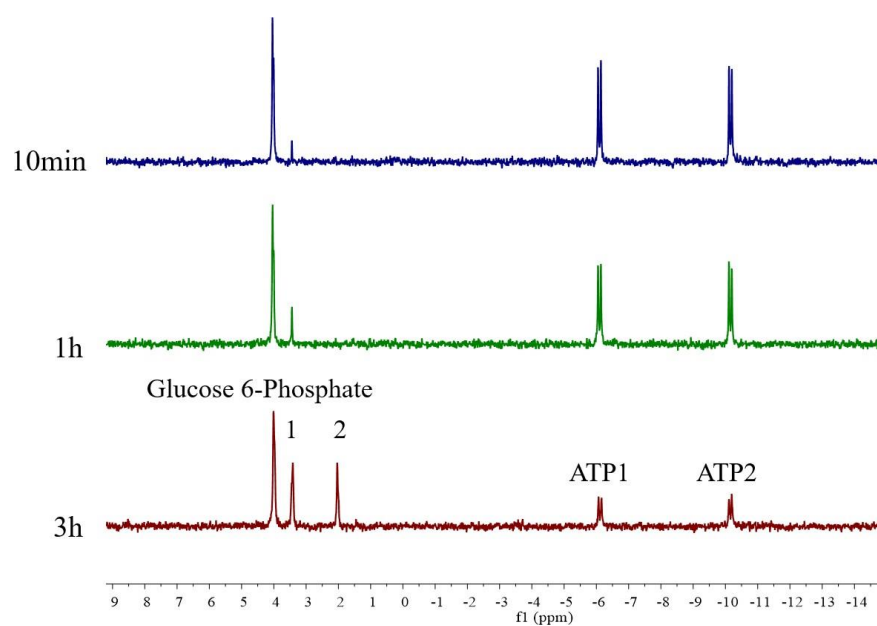## 6 Time gradient measurement of HK enzyme activity

Fig. S7. Enzymatic activity time gradient assay for HK. We analyzed the enzymatic activity of HK in real-time by the production of glucose-6-phosphate. Under our experimental conditions, a reaction time of 3 hours was sufficient to produce detectable amounts of glucose-6-phosphate. Moreover, the enzymatic reaction produces many by-products, but the chemical shifts are all different from glucose-6-phosphate and can be well distinguished.
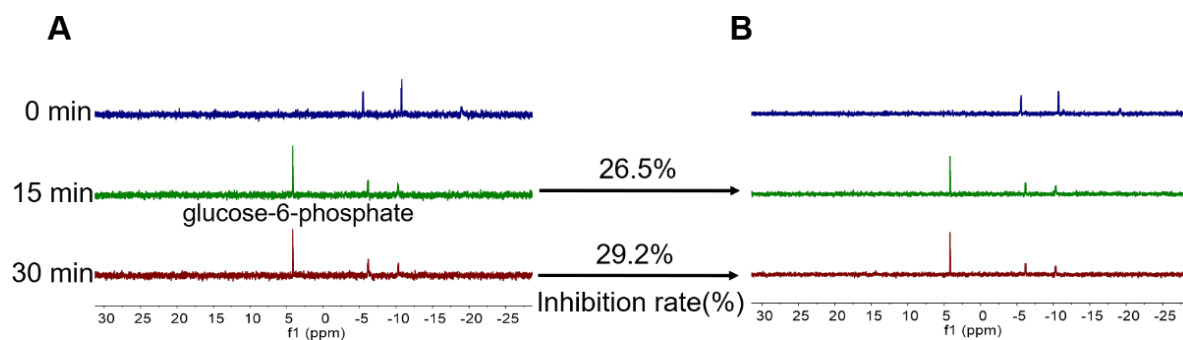
7 ³¹P NMR screening of inhibitors

Fig. S8. ³¹P NMR screening for the HK inhibitor Lonidamine. Real-time 31P NMR spectra of glucose-6-Phosphate products catalyzed by HK (0.5 mg mL-1) in the presence of matching DMSO (A) or 200 μM inhibitor Lonidamine (B).