# Luciferase-based assay for adenosine: Application to *S*-adenosyl-L-homocysteine hydrolase.

Emmanuel S. Burgos,[1] Shivali A. Gulab,[1,2] María B. Cassera[3] and Vern L. Schramm[1]

[1]Department of Biochemistry, Albert Einstein College of Medicine of Yeshiva University, 1300 Morris Park Avenue, Bronx, New York 10461, United States, [2]Carbohydrate Chemistry Team, Industrial Research Limited, PO Box 31-310, Lower Hutt, New Zealand, [3]Department of Biochemistry, Virginia Tech, Blacksburg, VA 24061, United States

## Supporting Information

## 1. Supplementary Methods

**Phosphorylation of neplanocin A by *Ag*AK.** Analyses were performed by HPLC on a reverse phase $C_{18}$ column (Luna[2], 150×4.6 mm, 5 μm, 100 Å; phenomenex) using a protocol previously described;[1] ADO, AMP, GDP, GTP, SAH, INH and INH-P could be easily separated and our analyses demonstrate that INH is a substrate of *Ag*AK according to the formation of GDP.

**SAHH inhibition by neplanocin A (INH).** In absence of *Ag*AK, two experiments were performed in parallel. The SAHH enzyme mixture (100 mM TRIS-acetate pH 7.7, 0.5 mM PEP, 0.5 mM PPi, 2 mM ammonium chloride, 10 mM $MgCl_2$, 0.5 mM GTP, 1 mM THP and 250 mU $L^{-1}$ of SAHH) was rapidly added to an equal volume of SAH (40 μM; with or without 12 μM INH). Samples were taken every ten minutes, quenched by ultrafiltration (Biomax UltraFree 0.5 mL centrifugal filter, 10 KD NMWL; Millipore) and analyzed by HPLC to follow ADO production. In presence of the same kinase, two similar experiments were carried; the SAHH mixture (see above; supplied with 60 U $L^{-1}$ of *Ag*AK) was added to an equal volume of SAH (40 μM; with or without 12 μM INH). Every ten minutes, samples were processed as described above to quantify the formation of AMP.

## 2. Supplementary Figures

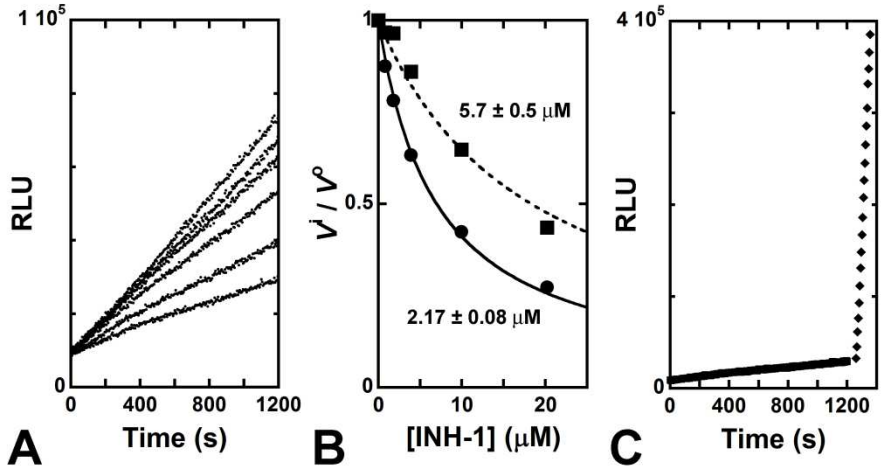


**Figure S1.** $K_i$ evaluation for 5′-deoxy-5′-amino-$\beta$-D-adenosine (INH-1). **A**) Light output profile monitored for 20 minutes at 20, 10, 4, 2 and 0.8 μM of INH-1 (*cf.* Experimental Section). **B**) INH-1 displays a slight slow-onset inhibition (dashed trace) with a $K_i^*$ of 2.17 ± 0.08 μM. **C**) Assay control for molecules displaying micromolar $K_i$s. At the end of the inhibition experiment with INH-1 (20 μM), ADO was added to a 50 μM final concentration and luminescence was monitored; the coupling enzymes AK/PPDK/FLUC are not inhibited by high concentrations of INH-1 (diamonds signs).

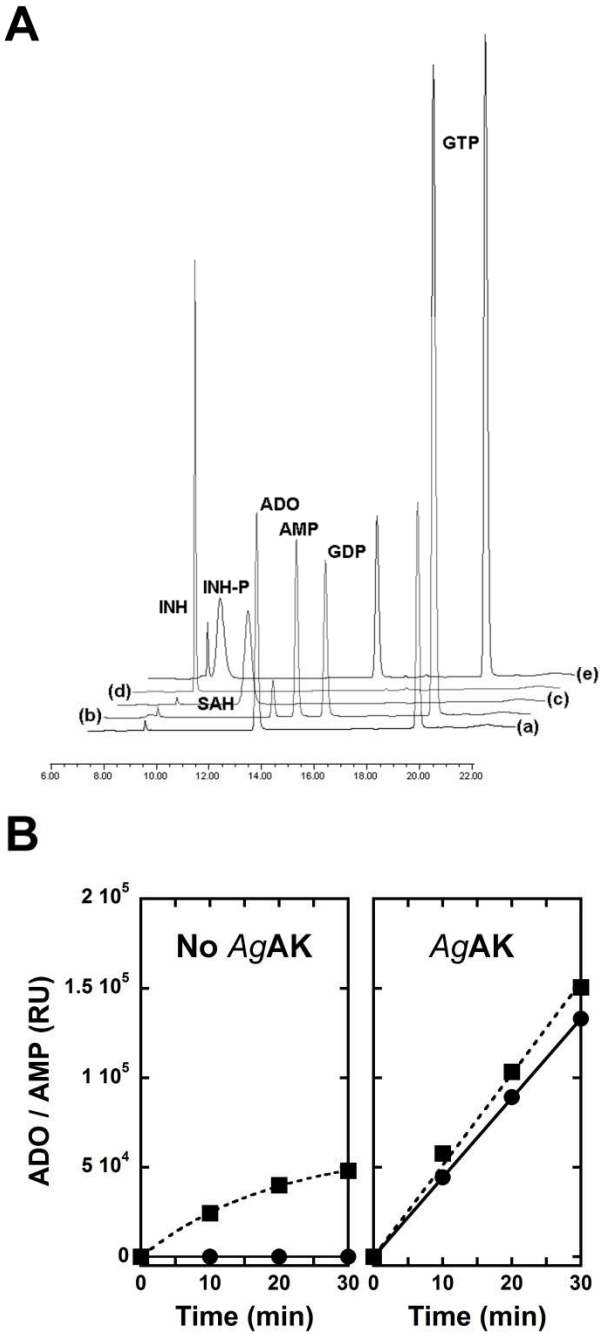


**Figure S2.** The phopshorylation of neplanocin A by *Ag*AK and its implications for future screenings with our luciferase-based assay. **A**) HPLC analysis of species encountered during the assay. ADO and GTP (Trace a) are converted to AMP and GDP (Trace b) upon addition of *Ag*AK; starting materiel SAH, substrate of SAHH (Trace c) and neplanocin A (Trace d); according to the production of GDP, neplanocin A (INH) is a substrate of *Ag*AK and get phosphorylated into INH-P (Trace e). **B**) Under conditions where *Ag*AK is omitted (left panel), INH (6 μM) fully inhibits the production of ADO by SAHH (100 nM; solid trace, round signs) in presence of SAH (20 μM). However, INH gets fully phosphorylated when the kinase is added to the enzyme mixture (30 mU $mL^{-1}$ final concentration) and there was no inhibition observed (right panel). The AMP production for the control (no inhibitor; dashed line, square signs) and the measure experiments (with inhibitor; solid line, round signs) are nearly identical.

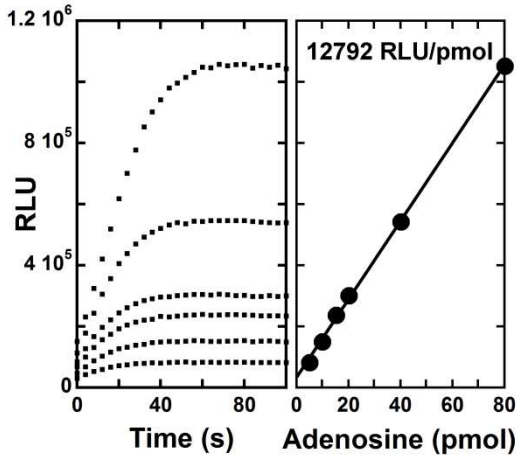


**Figure S3.** Effect of DMSO on the chemiluminescence output. A new ADO calibration was established in presence of DMSO (1.75%, v/v) using the typical experimental conditions; the linear response display the same sensitivity as our previous calibration experiment where DMSO was omitted (12792 RLU $pmol^{-1}$ vs. 12570 RLU $pmol^{-1}$). Thus, the coupling enzymes AK/PPDK/FLUC are not affected by this organic solvent.

## 3. Supplementary References

(1) Burgos, E. S.; Schramm, V. L. *Biochemistry* **2008**, *47*, 11086-11096.