Electronic Supplementary Material (ESI) for ChemComm.

Supporting information

An NMR Based Phosphodiesterase Assay

Short title: **PDE NMR Assay**

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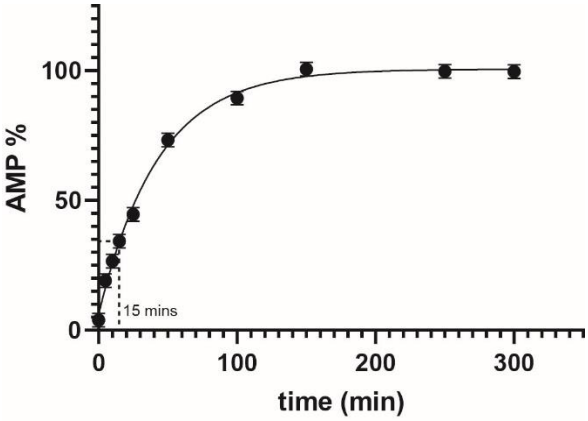

Fig. S1 Real time cAMP hydrolysis by PDE8A (5 nM), plot of H8 AMP % *vs.* reaction time. The Fig. 1C calibration curve was used to measure the % AMP.

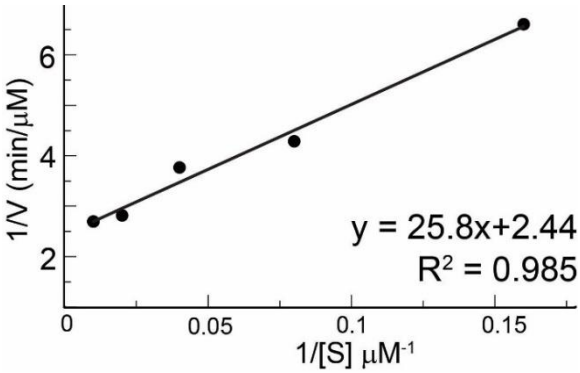

Fig. S2 The Lineweaver-Burke plot of Fig. 2C from which a K_M of 10.6 ± 1.6 μM and a V_{max} of 0.41 ± 0.01 μM/min were determined.

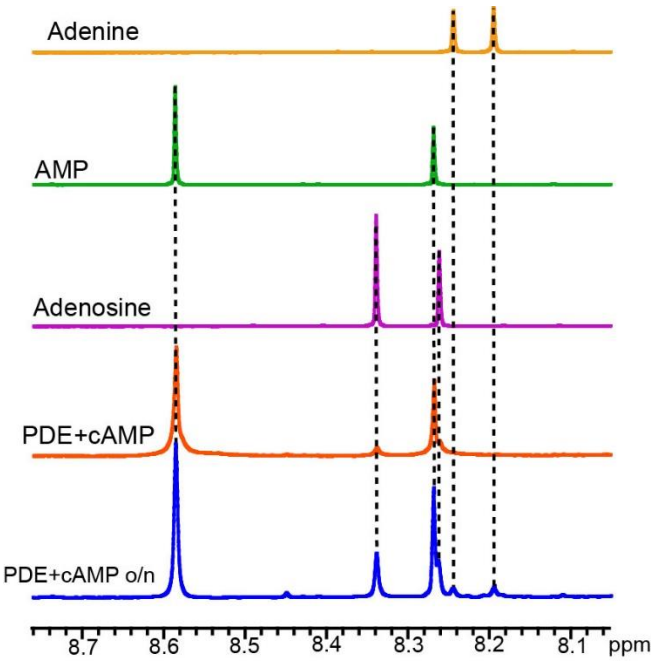

Fig. S3 PDE8A was added to 0.2 mM cAMP and the reaction was continued in the NMR spectrometer at 306K. The ^{1}H NMR spectra clearly detect AMP produced by the cAMP hydrolysis (red). After overnight reaction, further hydrolysis of AMP was observed (blue). The final PDE8A concentration was 15 μM. Reference ^{1}H NMR spectra of AMP (green), adenine (orange) and adenosine (purple) were acquired as standards.

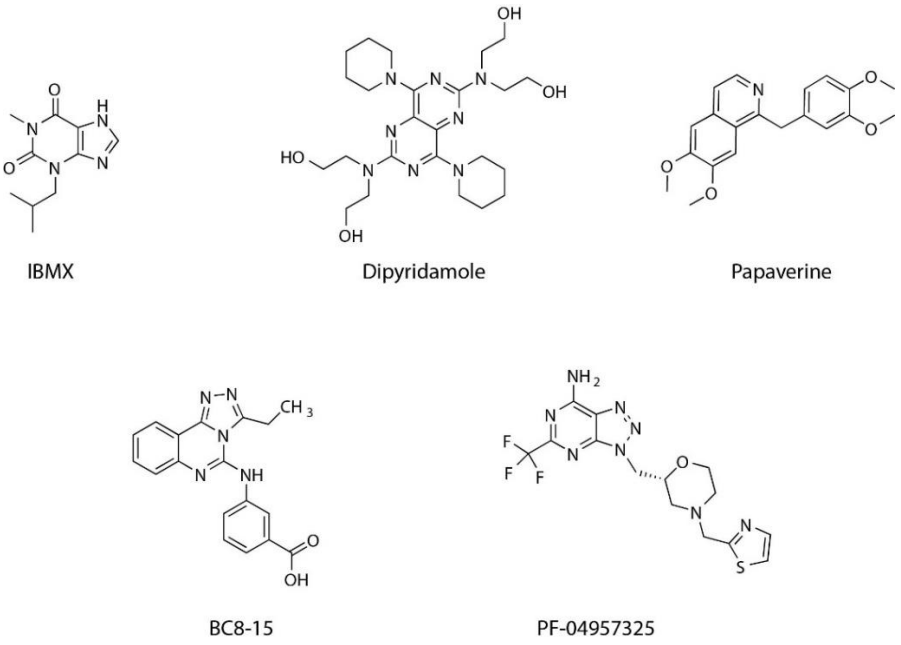

Fig. S4 PDE inhibitors analyzed in this manuscript.[31-33]

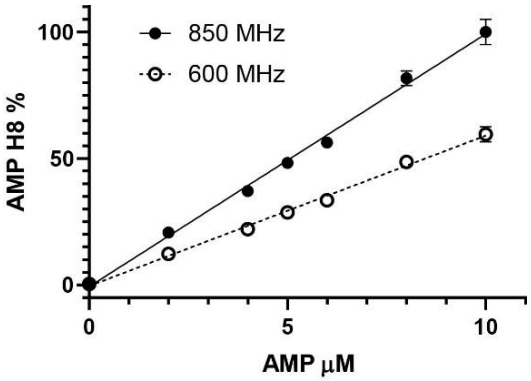

Fig. S5 Signal to noise expected for H8 AMP at 600 MHz and experimental results at 850 MHz. The signal intensity of 10 μM AMP H8 at 850 MHz is normalized to 100% AMP H8.

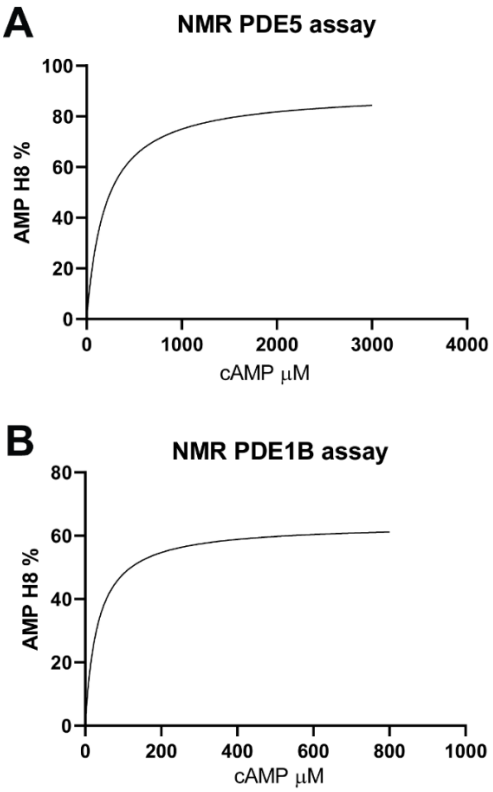

Fig. S6 Simulated Michaelis-Menten plots of PDE-catalyzed cAMP hydrolysis based on published kinetic parameters (K_M and Vmax)[34]. The reaction time was set at 15 mins in all simulations. The calibration curve in Fig. 1C is used to compute the % AMP concentration. A) Michaelis-Menten plot expected for PDE5 (K_M = 200 μM; V_{max} = 20 μmol/min/mg). PDE5 and cAMP concentrations of 0.3 nM and 0-3 mM, respectively, were used for these simulations. B) Michaelis-Menten plot expected for PDE1B (K_M = 33 μM; V_{max} = 1.5 μmol/min/mg). PDE1B and cAMP concentrations of 5 nM and 0-0.8 mM, respectively, were used for these simulations. This figure illustrates that the K_M values dictate the cAMP concentration range, while the V_{max} values affect primarily the choice of PDE concentration.

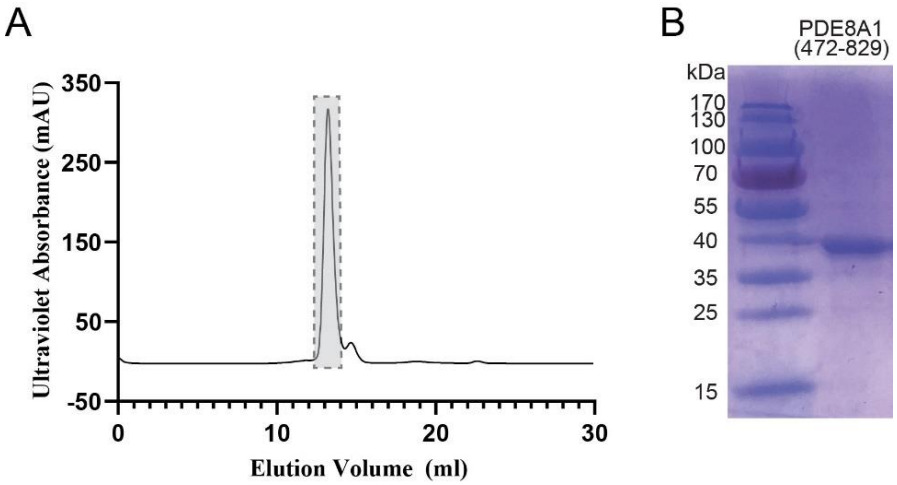

Fig. S7 A) Size exclusion chromatography profile of PDE8A1 (472-829) with Superdex 200 increase 10/300 column. The gray box highlights the collected fraction of the PDE used for the NMR assay. B) SDS-PAGE of purified PDE8A1 (472-829) stained with Coomassie Brilliant Blue. Lane 1, marker proteins; Lane 2, purified catalytic domain of PDE8A1.

Experimental Procedures

Protein purification: The catalytic domain of PDE8A1 472-829 (PDE) was expressed in BL21(DE3) cells and purified as previously reported[35] (Fig. S7). This PDE8A1 construct is referred to here interchangeably as PDE or PDE8A.

Accuracy assessment through AMP control experiments: ^{1}H NMR spectra were acquired with different AMP concentrations in 20 mM Tris-HCl, pH 7.5, 10 mM $MgCl_2$ and 1 mM DTT with 10% D_2O. Proton 1D NMR were acquired on a Bruker 850 MHz Avance III spectrometer equipped with a room temperature TXI probe, at 306K using a 3-9-19 pulse sequence with NS 128 scans and DS as 16 scans. SW was15.98 ppm and TD points were 32768. Data were processed Using a squared sine-bell window function with a shift factor of 2 before Fourier transform. The intensity of the peak corresponding to the H8 AMP proton was measured to determine the relative amount of AMP produced by the phosphodiesterase-catalyzed hydrolysis of cAMP. The calibration curve (Fig 1C) was used to measure the % AMP. If line-broadening is observed, an exponential multiplication window function with an LB factor significantly greater than the line-width is recommended to minimize biases on the relative intensities. Alternatively, if the intensity does not correspond to the amount of material present, integrals are preferred.

Real-time NMR monitoring of PDE-catalyzed cAMP hydrolysis: PDE 5 nM, cAMP 10 μM were added in 20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$ and 1 mM DTT with 10% D_2O on ice. The enzymatic reactions were carried at 306K and monitored by real time NMR with 128 scans and 16 dummy scans, a spectra width of 15.98 ppm and 32K points per consecutive 1D NMR spectrum. The reduction of cAMP and the increase of AMP was monitored by measuring the changes in the intensity of H8 cAMP and H8 AMP, respectively.

Determination of kinetic parameters: The purified PDE8A1 (5 nM) was incubated for 15 mins at room temperature with 20 mM Tris–HCl, pH 7.5, 1 mM DTT, 10 mM $MgCl_2$ and various concentrations of cAMP. The reaction was terminated by boiling the sample at 95 °C, vortexing and pelleting the PDE by centrifugation. NMR confirmed that further hydrolysis was indeed quenched. The enzymatic reaction product, AMP, was quantified by 1D ^{1}H NMR. Six concentrations of cAMP in a range of 0.02–100 μM were used to obtain the kinetic parameters K_M and k_{cat}. Non-linear regression of the Michaelis–Menten equation and Lineweaver Bruke plots were used for the data analysis.

Inhibitor screening: PDE 10 nM, cAMP 10 μM and 10 μM dipyridamole, PF-0457325, IBMX, BC8-15, papaverine or 0.5 % DMSO were added in 20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$ and 1 mM DTT with 10% D_2O. Experiments were carried out for 10 mins at room temperature. Reactions were terminated by boiling at 95 °C for 1 minute and centrifugation. The boiling step stops the reaction by denaturing the PDE, which aggregates and sediments upon centrifugation. Proton 1D NMR were acquired on a Bruker 850 MHz Avance III spectrometer at 306K using a 3-9-19 pulse sequence with 128 scans. Data were processed Using a squared sine-bell window function with a shift factor of 2 before Fourier transform. Peaks corresponding to 10 μM H8 AMP were integrated to measure the AMP percentage.

Real-time NMR based Inhibitor Screening: PDE 10 nM, cAMP 10 μM and 200 μM dipyridamole, IBMX or 1% DMSO were added in 20 mM Tris-HCl pH 7.5, 10 mM $MgCl_2$ and 1 mM DTT with 10% D_2O on ice. The enzymatic reactions were carried at 306K and monitored by real time NMR with the same 1D ^{1}H NMR acquisition parameters indicated above. The reduction of cAMP

and the increase of AMP was monitored by measuring the changes in the intensity of H8 cAMP and H8 AMP, respectively.