



OPEN

A bioluminescent and homogeneous assay for monitoring GPCR-mediated cAMP modulation and PDE activity

Dareen Mikheil¹, Matthew A. Larsen², Kevin Hsiao¹, Nathan H. Murray¹, Tim Ugo², Hui Wang² & Said A. Goueli¹✉

3',5'-Cyclic adenosine monophosphate (cAMP), the first identified second messenger, is implicated in diverse cellular processes involving cellular metabolism, cell proliferation and differentiation, apoptosis, and gene expression. cAMP is synthesized by adenylyl cyclase (AC), which converts ATP to cAMP upon activation of G_{αs}-protein coupled receptors (GPCRs) in most cases and hydrolyzed by cyclic nucleotide phosphodiesterases (PDEs) to 5'-AMP. Dysregulation of cAMP signaling is implicated in a wide range of pathophysiological conditions such as cardiovascular diseases, neurodegenerative and behavioral disorders, cancers, diabetes, obesity, cataracts, and others. Therefore, cAMP targeted therapies have been and are still undergoing intense investigation for the treatment of these and other diseases. This highlights the need for developing assays to detect and monitor cAMP levels. In this study, we show cAMP Lumit assay as a highly specific homogeneous bioluminescent assay suitable for high throughput screenings with a large assay window and a wide dynamic range for cAMP detection. We believe that this assay will aid and simplify drug discovery screening efforts for cAMP signaling targeted therapies.

3',5'-Cyclic adenosine monophosphate (cAMP), the first identified second messenger, is synthesized by adenylyl cyclase (AC) which converts ATP to cAMP upon activation of G_{αs}-protein coupled receptors (GPCRs) in most cases^{1,2}. Cyclic nucleotide phosphodiesterases (PDEs) on the other hand degrade cAMP to 5'-adenosine monophosphate (5'-AMP). The rates of cAMP synthesis by AC and hydrolysis by PDEs determine the level of cAMP in the cell³. cAMP, as a second messenger, is essential for signal transduction as it transmits and amplifies signals coming from cell surface receptors⁴. Downstream of cAMP are Protein Kinase A (PKA), the guanine-nucleotide-exchange factor (GEF) EPAC, cyclic nucleotide-gated ion channels and Popeye domain containing proteins as main effectors of cAMP signaling⁵⁻⁸. The cAMP signaling pathway is entailed in diverse cellular processes involving cellular metabolism⁹⁻¹¹, cell proliferation and differentiation¹², apoptosis^{13,14}, and gene expression¹⁵.

Dysregulation of cAMP signaling has been found to be associated with several pathophysiological conditions such as cardiovascular diseases^{16,17}, neurodegenerative and behavioral disorders¹⁸⁻²¹, cancers^{22,23}, diabetes^{24,25}, obesity²⁶, cataracts²⁷, and others. The broad spectrum of these conditions and their prevalence worldwide highlights the need for continued efforts in cAMP signaling research for targeted therapies. Several studies have investigated targeting the cAMP/PKA pathway for cancer treatment^{28,29}, as well as diabetes³⁰, cardiovascular, kidney³¹ and other diseases. Targeting the cAMP pathway can be achieved mainly by cAMP level modulation and PKA inhibition. Some of the FDA-approved drugs work by modulating cAMP levels. An example is liraglutide and exenatide which are GLP1RR (GPCR) agonists used to treat diabetes. They trigger insulin secretion by stimulating β cells to produce cAMP. Also, PDE4 inhibitors have been developed that enhance glucose homeostasis in patients³². In the case of acute heart failure, colforsin daropate hydrochloride, an AC5-selective forskolin (FSK) derivative, activates AC5 to induce cAMP production³³. For patients with COPD experiencing severe air flow limitations, symptoms of chronic bronchitis, and a history of exacerbations roflumilast (a PDE4 inhibitor) is used as an FDA approved drug^{34,35}. Another FDA approved PDE4 inhibitor is apremilast used for psoriasis and psoriatic arthritis patients.

¹Promega Corporation, 2800 Woods Hollow Road, Madison, WI 53711, USA. ²Promega Biosciences, 277 Granada Drive, San Luis Obispo, CA 93401, USA. ✉email: said.goueli@promega.com

A number of assays are available for the detection and measurement of cAMP levels in both biochemical and cellular systems. Many of these assays are homogeneous time resolved fluorescence (HTRF)-based that use cAMP specific antibodies which bind to free cAMP or a fluorescently labeled cAMP conjugate (competitor). Fluorescently labeled cAMP binding to the antibody is then detected either by fluorescence resonance energy transfer (FRET) or enzymatic reactions. In addition, there are two assays from Promega for the detection of cAMP. The first one is the GloSensor cAMP assay which uses a split luciferase that reassembles upon cAMP binding. The downsides for this assay are mainly the need for cell engineering and the inability to run calibration curves using a cAMP standard. The other assay is the cAMP Glo bioluminescent assay; a very simple assay and was designed to address the limitations of the GloSensor assay, but it has a limited dynamic range for cAMP detection. Of these assays, the most commonly used for cAMP detection are the HTRF assays; however, fluorescence-based assays require fluorescence detection capable instruments that might not be readily available in every laboratory. In addition, in high throughput screening experiments, some compounds might have fluorescent properties which would interfere with the assay signal and performance and lead to false positives. Furthermore, the assay suffers from what is called the hook effect when the amount of antibody is limited. Bioluminescent assays on the other hand overcome these problems associated with fluorescent assays^{36,37}.

In this report, we show the utilization of the NanoLuc Binary Technology (NanoBiT) system³⁸ with a cAMP specific antibody and a cAMP tracer to detect cAMP in biochemical enzymatic as well as cellular assays. The assay is homogeneous and specific with a large assay window and a wide dynamic range for cAMP detection. The assay is suitable for high throughput screening (HTS) and can be run in different screening sizes on any simple luminometer. We believe that this assay will aid in simplifying screening efforts in drug discovery for cAMP signaling targeted therapies.

Materials and methods

cAMP-small BiT (SmBiT) tracer synthesis

cAMP-PEG3-SmBiT tracer synthesis methods are provided in detail in the supplementary data section.

Specificity testing and Z' factor determination

cAMP (Promega, V6421), 2'3'-cyclic adenosine monophosphate (2'3'-cAMP, Sigma A9376), cyclic guanosine monophosphate (cGMP, Promega V6411), 2'3'-cyclic guanosine monophosphate-adenosine monophosphate (2'3'cGAMP, Cayman Chemical 19887) and 3'3'-cyclic guanosine monophosphate-adenosine monophosphate (3'3'cGAMP, Sigma SML1232) were serially diluted (3-folds) starting at 10 μ M in 1 \times Immunoassay Buffer C (IAB-C, Promega VB115B). 20 μ l of the diluted metabolites were added to wells in a 96 well plate (Corning 3912). Antibody mix containing 1 ng Anti-cAMP/5 μ l (GenScript A01509), 15 nM Tracer (final concentration in the reaction), 3:500 diluted Lumit Anti-Mouse Ab-LgBit (Promega W102B) in 2 \times IAB-C was prepared. 20 μ l of the antibody mix were added to wells containing metabolites. Plates were mixed on an orbital shaker for 5 min (min) at 400 rpm then incubated for 30 min at room temperature. Nano-Glo Luciferase Assay Substrate (Promega N113C) was prepared by diluting the stock solution 12.5-fold in 1 \times IAB-C. 10 μ l of diluted substrate were then added to reaction wells. Plates were mixed on an orbital shaker for 3 min at 400 rpm before measuring luminescence signal on a GloMax Discover Microplate Reader (Promega GM3000) within 15 min. Metabolite concentrations were reported as initial concentrations in the 20 μ L prior to antibody mix addition to the wells.

For competition experiments, a similar protocol was followed except that the initial 20 μ l of the diluted metabolite in the reaction was divided into 10 μ l of 2X diluted cAMP and 10 μ l of 2X diluted competitor metabolite (or buffer for control).

For Z' factor determination, a similar protocol was followed except that cAMP was diluted to 1 μ M, 0.5 μ M, 0.25 μ M, 0.1 μ M, 0.05 μ M and 0.025 μ M. The assay was run for cAMP at each concentration alongside a buffer control for comparison (48 replicates each, on the same plate). Z' factor values were calculated according to the previously described method (briefly described below in the statistics methods section).³⁹

PDE4 inhibition study

PDE4A1A enzyme (BPS Biosciences 60040) was diluted to 2 ng/ μ L in 1XPDE Reaction Buffer (40 mM Tris pH 7.5, 10 mM MgCl₂, 0.1 mg/mL BSA). 10 μ L were added to wells in a 96 well plate. Ro-20-1724 or Difamilast (TargetMol T27172), PDE4 inhibitors, were serially diluted in 1XPDE Reaction buffer keeping dimethyl sulfoxide (DMSO, Sigma D2650) concentration equal between all dilutions. 10 μ L of diluted inhibitor were added to wells containing the enzyme. Plates were mixed on an orbital shaker for 10 min at 400 rpm. 10 μ L of 30 μ M cAMP (final 10 μ M in the 30 μ L) in 1XPDE Reaction Buffer were added as the substrate needed to initiate the reactions. Plates were mixed on an orbital shaker for 5 min at 400 rpm and then incubated for another 25 min at room temperature. 20 μ L of 1.25 mM IBMX in 1XPDE Reaction Buffer were added to wells to terminate reactions. Plates were mixed on an orbital shaker for 5 min at 400 rpm. Each well then received 50 μ L of the antibody mix containing 1 ng Anti-cAMP/5 μ l, 15 nM Tracer (final concentration in the reaction), 3:500 diluted Lumit Anti-Mouse Ab-LgBit in 2 \times IAB-C and plates were mixed for 5 min at 400 rpm and incubated for 30 min at room temperature. Nano-Glo Luciferase Assay Substrate was prepared by diluting the stock solution 12.5-fold in 1 \times IAB-C. 25 μ L of the diluted substrate were then added to each well. Plates were mixed on an orbital shaker for 3 min at 400 rpm. Luminescence signal was detected on a GloMax Discover Microplate Reader within 15 min. Inhibitor concentrations were reported as initial concentrations in the 30 μ L enzyme reaction before excess IBMX were added to terminate reactions. Percent inhibition was determined by normalizing to a no inhibitor control.

Cell based assays

HEK293, A375, DRD1 stably transfected HEK293 and DRD2 stably transfected HEK293 cells were maintained in Dulbecco's Modified Eagle Medium (DMEM, Gibco 11995-065) + 10% fetal bovine serum (FBS, Avantor 89510-194) + 1% Penicillin–Streptomycin (Gibco 15140-122). Media used for stably transfected cells was further supplemented with 500 µg/mL of Genetecin (Gibco 10131035). Cells were trypsinized (Gibco 25200-056) and counted using an automated cell counter (BioRad TC20). Cells were plated in 384 well plates (Corning 3570) at 1000 cells/well in 25 µL media and allowed to adhere and recover overnight at 37 °C/5% CO₂. The next day, compounds and cAMP standard were serially diluted in Hank's Balanced Salt Solution (HBSS, Gibco 14025-092) containing 500 µM 3-isobutyl-1-methylxanthine (IBMX, Sigma I5879) and 100 µM Ro 20-1724 (Sigma B8279). Media was removed from wells and 5 µL of the diluted compounds were added to each well. 5 µL of diluted cAMP were added to wells without cells for standard curve. Plates were mixed on an orbital shaker for 5 min at 400 rpm. Treatment incubation (at room temperature) times as follows: 1 h for Dopamine (Sigma H8502), SKF38393 (Sigma D047), SCH23390 (Sigma 505723), Quinpirole (Sigma Q102), L741,626 (Tocris 1003) and NECA (Sigma I19140); 30 min for FSK (Sigma F6886); 5 min for Isoproterenol (Tocris 1747), epinephrin (Sigma 1236970), Propranolol (Sigma P0884) and Alprenolol (Sigma A0360000). 5 µL of 2% trichloroacetic acid (TCA, Fisher Scientific SA9410-500) in water were added to wells to terminate reactions and lyse cells. 5 µL of 2% TCA were also added to cAMP wells. Plates were mixed on an orbital shaker for 5 min at 400 rpm. 5 µL of the antibody mix containing 2 ng Anti-cAMP/5 µL, 15 nM Tracer (final concentration in the reaction) and 3:500 diluted Lumit Anti-Mouse Ab-LgBiT in 0.25X IAB-C/0.3M Tris, pH 9.0 were added to each well. Plates were mixed on an orbital shaker for 5 min at 400 rpm then incubated for 30 min at room temperature. 5 µL of Nano-Glo Luciferase Assay Substrate diluted 26-fold in 0.25 × IAB-C were then added to each well and plates were mixed on an orbital shaker for 3 min at 400 rpm. Luminescence signal was measured on a GloMax Discover Microplate Reader within 15 min. Reported compounds concentrations are concentrations in the initial 5 µL treatment before TCA addition, while reported cAMP concentrations are final concentrations in the reaction.

Software and statistics

For Z' factor calculations and statistical analyses Excel software was used ($Z' = 1 - \frac{3*(SD_s + SD_c)}{|\mu_s - \mu_c|}$); in this equation μ = mean, SD = standard deviation, s = sample and c = control. For standard curves, inhibitor studies and cellular assays statistical calculations and figures generation GraphPad Prism 9.1.0. was used to determine the EC₅₀/IC₅₀ using Nonlinear regression (curve fit) using sigmoidal dose response. In all instances n = the number of independent sample replicates within an experiment and SD indicates the standard deviation.

Results and discussion

Assay principle and format

The cAMP Lumit immunoassay system is a homogeneous competitive assay for detecting and monitoring cAMP in biochemical enzymatic reactions or in cellular systems. Figure 1 shows a schematic representation of the cAMP Lumit assay. Similar to other Lumit technologies⁴⁰, this assay relies on the NanoLuc Binary Technology (Nano-BiT)³⁸. In a NanoBiT system, a structural complementation between the Large BiT (LgBiT) luciferase subunit and the Small BiT (SmBiT) peptide, when brought into close proximity, results in the formation of a functional enzyme that generates luminescence, in the presence of its substrate³⁸, that is proportional to the extent of NanoBiT complementation. In this assay, a cAMP-SmBiT tracer molecule that resembles the endogenous cAMP is employed along with a cAMP specific primary antibody and a secondary antibody conjugated to the LgBiT subunit. If there is no cAMP present in the reaction, the cAMP-SmBiT tracer complements the LgBiT subunit forming functional luciferase enzyme which produces light in the presence of its substrate. On the other hand,

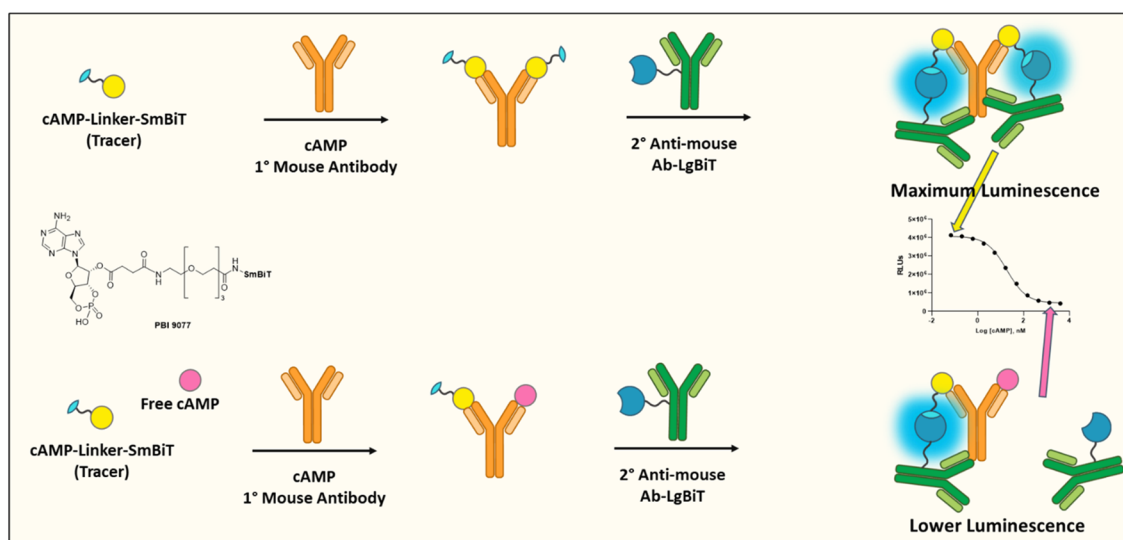


Figure 1. Schematic representation of the cAMP Lumit assay principle.

if there is cAMP in the reaction, it binds to the antibody competing with the cAMP-SmBiT tracer. This results in a lower level of NanoBiT complementation that is manifested in a lower bioluminescence signal.

Assay development

To build the cAMP Lumit assay, a functional antibody-tracer pair was necessary. We found the combination of an anti-cAMP primary antibody from GenScript (A01509) with a cAMP-SmBiT tracer containing a PEG3 linker between the cAMP and the SmBiT peptide showed the highest performance. In the presence of 10 μ M cAMP (final concentration), this combination consistently resulted in more than 25-fold lower signal compared to the no cAMP control (Assay window). Figure 2A shows a dose dependent response to a cAMP standard (EC_{50} = 9.6 nM cAMP). The assay range in this example was determined using the EC_{10} = 0.85 nM of cAMP and the EC_{90} = 106.9 nM of cAMP (EC_{10} – EC_{90} = 106.1 nM of cAMP). Figure 2B shows the same cAMP dose response data (shown in Fig. 2A) by plotting Δ RLUs against cAMP concentration. Figure 2C,D show the cAMP Lumit assay specificity. This was tested by titrating another form of cAMP (2'3'-cAMP) or potential cross-reactive metabolites (2'3'-cGAMP; 3'3'-cGAMP; cGMP) in the system, instead of cAMP. None of the tested metabolites resulted in a significant luminescence signal drop at concentrations up to 10 μ M. To test for the interference ability of these molecules with the assay, they were included in the assay at a concentration of 10 μ M. Nearly identical cAMP standard curves were generated showing no effect on the cAMP Lumit assay's ability to detect cAMP as shown in Fig. 2E,F. Hence, we concluded that the assay is specific, and that other cellular metabolites should not hinder its cAMP detection ability.

To determine the robustness and reproducibility of the cAMP Lumit assay, Z' factor values were calculated for 1, 0.5, 0.25, 0.1, 0.05 and 0.025 μ M of cAMP. A Z' factor of 0.5 or more (maximum 1) is interpreted as an excellent assay for high throughput screening. The luminescent signal in wells containing each of the mentioned cAMP concentrations was compared to buffer containing wells in a 96 well format (Fig. 3). For all the tested concentrations the Z' factor values were 0.67 or more. This data shows that the cAMP Lumit assay is both robust and reproducible which makes it suitable for high throughput screening.

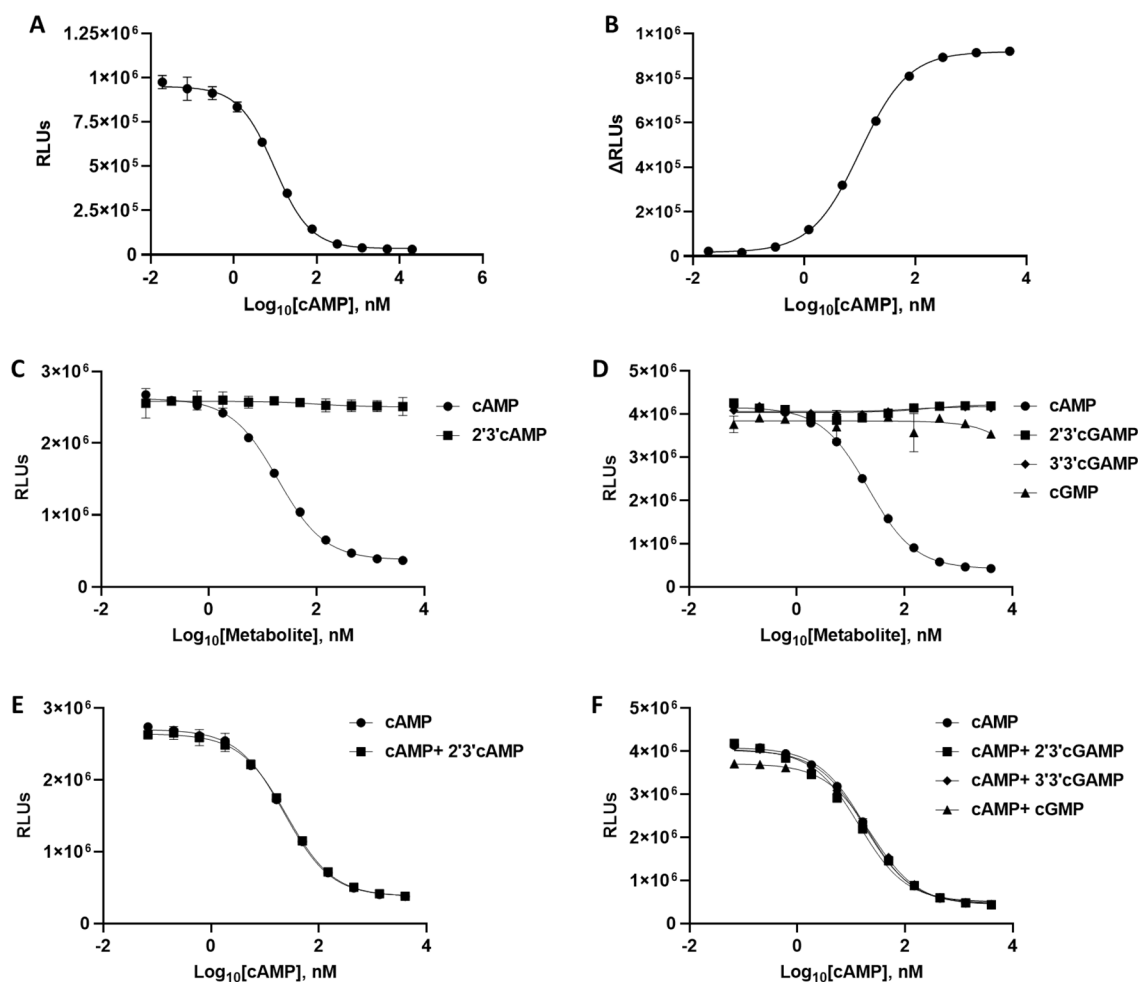


Figure 2. cAMP Lumit Assay range and specificity. (A,B) cAMP standard curve ($n=3 \pm \text{SD}$). (C,D) Titration of other metabolites in comparison with cAMP titration in cAMP Lumit assay ($n=2 \pm \text{SD}$). (E,F) 10 μ M of potential interfering metabolites (or buffer) were included in a cAMP titration assay ($n=2 \pm \text{SD}$).

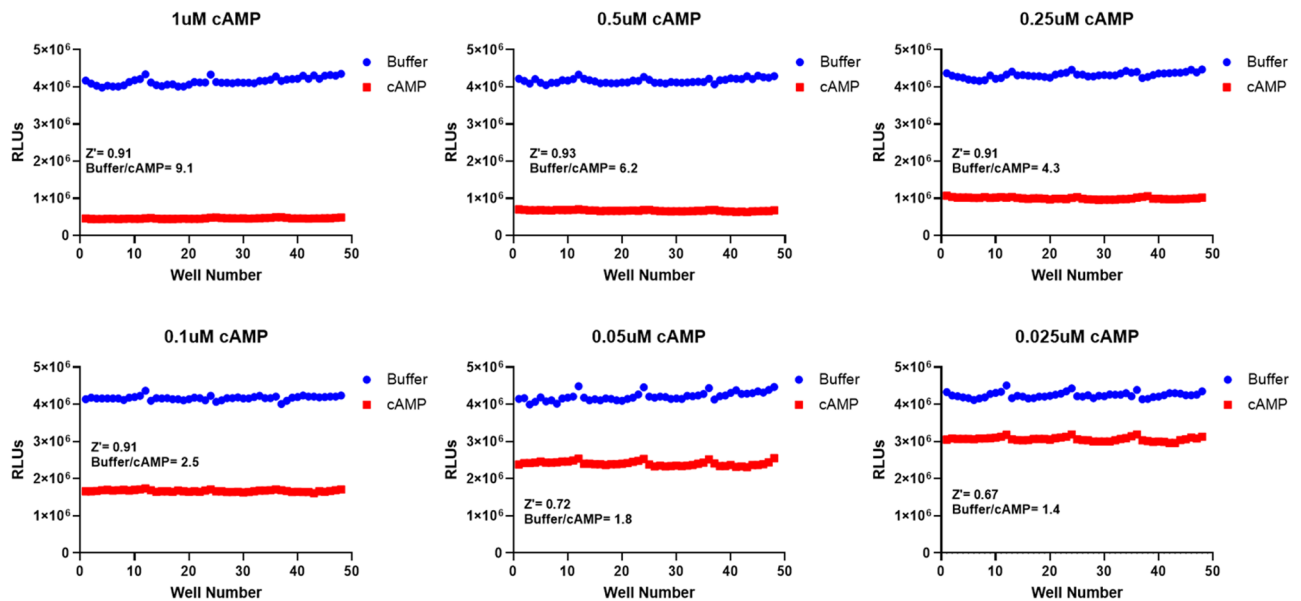


Figure 3. Z' factor test for the cAMP Lumit assay. cAMP Lumit assay was run for the indicated cAMP concentrations and compared to buffer controls to assess assay performance and calculate Z' factors at each concentration.

Measurement of PDE inhibition

PDEs constitute the main path through which cAMP is hydrolyzed. To date, 11 PDE families have been characterized. One way for PDE classification is substrate specificity. While PDE4, PDE7, and PDE8 are cAMP specific, PDE5, PDE6 and PDE9 are cGMP specific. On the other hand, PDE1, PDE2, PDE3, PDE10, and PDE 11 use both cAMP and cGMP as substrates^{41,42}. Three PDE4 inhibitors, apremilast, crisaborole, and roflumilast, are FDA approved and they are used for skin and lung diseases treatment⁴³ demonstrating the promise of targeting cAMP signaling. To show the value of our assay in PDE inhibition studies and targeting for drug discovery, we tested the PDE 4 inhibitors Ro 20–1724 and Difamilast in the cAMP Lumit assay. Our results showed that both Ro 20–1724 and Difamilast inhibit PDE4A1A in a dose dependent manner with IC_{50} = 416.3 nM and 7.3 nM, respectively (Fig. 4). This biochemical study indicates the suitability of the cAMP Lumit assay in monitoring PDE activity, which is of great value in drug discovery and development.

cAMP detection in cellular systems

As mentioned above, cAMP signaling mostly gets initiated through GPCRs, which constitute the largest protein family and are the most common target of FDA approved drugs. To date, about 134 GPCRs are targeted by approved drugs (more than 700 drugs), however none of these GPCRs are orphan receptors, which highlights the high potential for more discoveries and further studies in this area. Moreover, novel ways for targeting GPCRs continue to be under investigation aiming to generate different classes of drugs such as biased signaling drugs, allosteric modulators, antibodies, aptamers, gene therapy agents, and others. As such, the targeting of GPCRs and cAMP signaling will remain a hot area of research for drug discovery⁴⁴.

To demonstrate the applicability of cAMP Lumit in cellular assays, we manipulated cAMP levels in different cell lines using different GPCR agonists and antagonists (Table 1) and employed cAMP Lumit for the detection of cAMP levels changes in these cells. The assays were conducted by treatment of cells followed by lysis with 2% TCA, which also terminates the reactions. Cell lysates were then incubated with antibodies and tracer diluted in 0.3 M Tris buffer (pH 9.0; for neutralization) for 30 min after which time the detection reagent was added. All treatments resulted in a detectable change in cAMP levels by our cAMP Lumit Assay (Fig. 5). Even with the effect of TCA and Tris treatment on the assay background, reduction of luminescent signal ranged between 2.2 and 20-fold, maintaining a good assay window for cAMP detection in cellular systems. Taken together, the data presented here show that the cAMP Lumit assay is a competent assay that can be easily adapted for the detection of cAMP production in different cellular systems for the support of GPCR and cAMP-related drug discovery.

Conclusion

cAMP signaling is key for various cellular processes and it has been implicated in several diseases. The targeting of cAMP signaling for drug discovery is anticipated to continue to grow, which mandates the availability of a simple and reliable cAMP detection system. Commercially available cAMP detection assays either require cell engineering or need specialized plate readers capable of fluorescent signal detection or are ELISA-based. The cAMP Lumit assay demonstrated in this study, on the other hand is a simple, sensitive, specific, and direct assay that requires no cell engineering, which can get laborious and limiting for biosensor-based assays with some types of cells. In addition, cAMP Lumit has a broader dynamic range in comparison with other available assays,

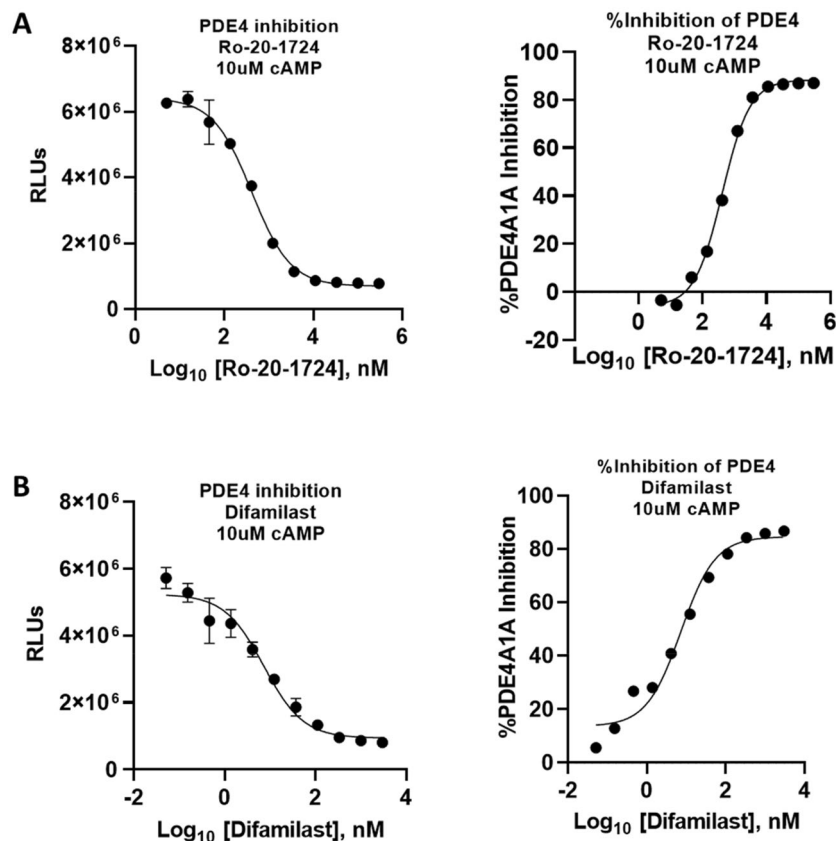


Figure 4. cAMP Lumit assay detects PDE inhibition. (A) Ro 20-1724, and (B) Difamilast. The right side of each figure shows the transformation of the data into percent inhibition of the data on the left side. Inhibitor titrations were carried out using 20 ng PDE4A1A per reaction in the presence of 10 μ M cAMP ($n = 2 \pm$ SD).

Receptor	G α -submit	Cell line	Agonist	Antagonist	Figure
Dopamine D1	Gas	HEK293 cells stably transfected with DRD1	Dopamine; SKF38393	SCH23390 in the presence of 100 nM SKF38	5A
Dopamine D2	Gai	HEK293 cells stably transfected with DRD2	Quinpirole in the presence of 5 μ M FSK	L741, 626 in the presence of 200 nM Quinpirole + 8 μ M FSK	5B
β -Adrenergic	Gas	A375 cells, endogenous receptor levels	Isopreterenol; Epinephrine	Propranolol; Alprenolol in the presence of 50 nM isopreterenol	5C
Adenosine	Gas	A375 cells, endogenous receptor levels	NECA	**	5D
**	**	HEK293	FSK	**	5D

Table 1. GPCR agonists and antagonists for cAMP levels manipulation.

only needs a simple luminometer and is not susceptible to interference with fluorescent compounds. Moreover, the assay is suitable for HTS and automation, a feature that ELISA-based assays lack. Our data show the assay's robustness and competence in both enzymatic biochemical and cellular assays which makes it the perfect choice for drug screening for cAMP signaling targeted therapies.

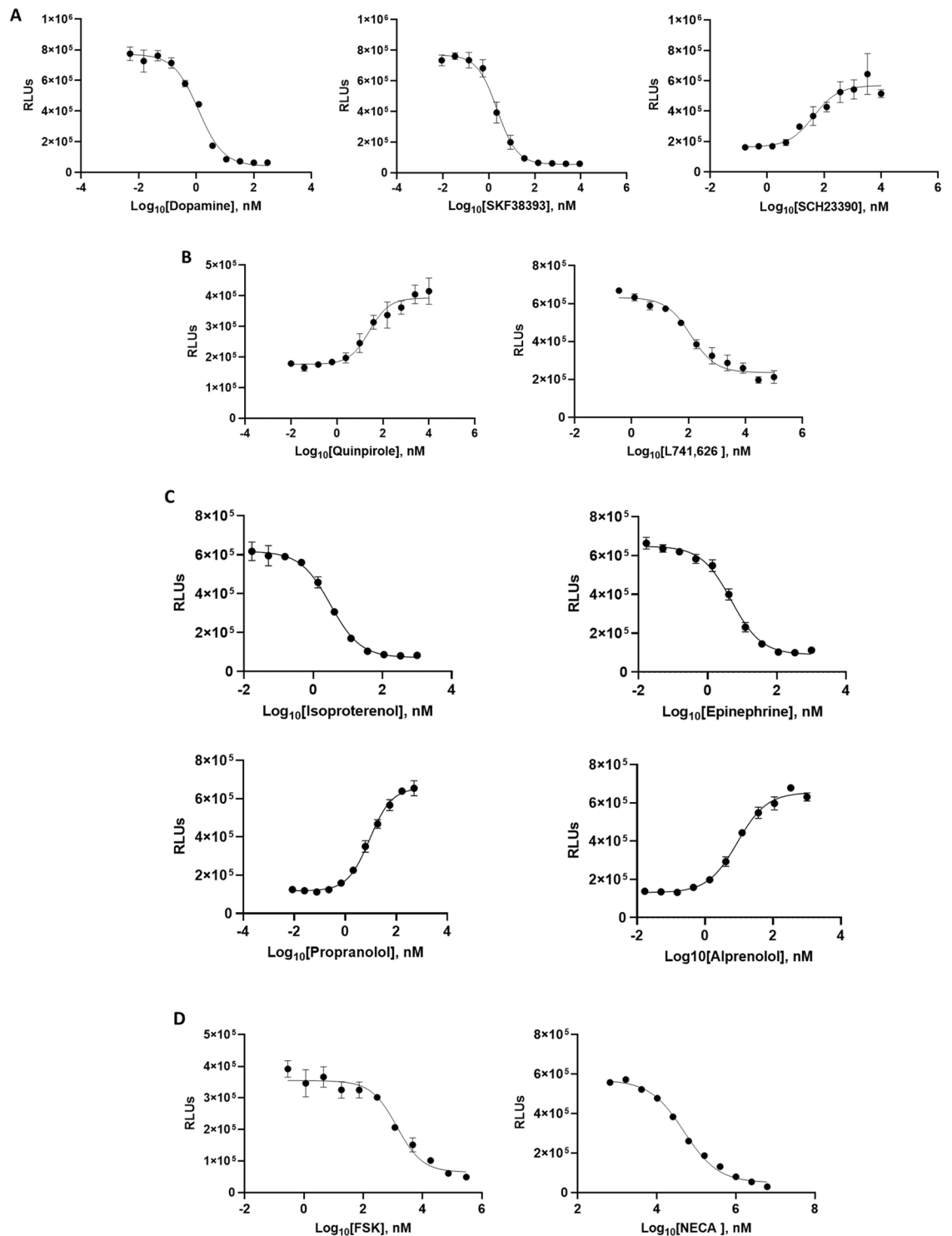


Figure 5. cAMP detection in cellular systems. (A) HEK293 cells stably transfected with DRD1 were treated with the DRD1 agonists Dopamine or SKF38393 leading to cAMP levels increase, or the DRD1 antagonist SCH23390 (in the presence of 100 nM SKF38393) leading to cAMP levels reduction ($n = 3 \pm SD$). (B) HEK293 cells stably transfected with DRD2 were treated with the DRD2 agonist Quinpirole (in the presence of 5 μ M Quinpirole + 8 μ M FSK) leading to cAMP levels reduction, or the DRD2 antagonist L741, 626 (in the presence of 200 nM Quinpirole + 8 μ M FSK) leading to cAMP levels increase ($n = 3 \pm SD$). (C) A375 cells were treated with the β -Adrenergic agonists Isopreterenol or Epinephrine leading to cAMP levels increase, or the β -Adrenergic receptor antagonists Propranolol or Alprenolol (in the presence of 50 nM Isopreterenol) leading to cAMP levels reduction ($n = 3 \pm SD$). (D) HEK293 cells were treated with Forskolin and A375 cells were treated with the Adenosine receptor agonist NECA, both leading to cAMP levels increase ($n = 3 \pm SD$).

Received: 30 October 2023; Accepted: 20 February 2024

Published online: 23 February 2024

References

- Sutherland, E. W. & Rall, T. W. Fractionation and characterization of a cyclic adenosine monophosphate formed by tissue particles. *J. Biol. Chem.* **232**(2), 1077–1091 (1958).
- Yau, K. W. Cyclic nucleotide-gated channels: an expanding new family of ion channels. *Proc Natl Acad Sci U S A* **91**(9), 3481–3483 (1994).
- Houslay, M. D. & Milligan, G. Tailoring cAMP-signalling responses through isoform multiplicity. *Trends Biochem. Sci.* **22**(6), 217–224 (1997).
- Francis, S. H., Blount, M. A. & Corbin, J. D. Mammalian cyclic nucleotide phosphodiesterases: Molecular mechanisms and physiological functions. *Physiol. Rev.* **91**(2), 651–690 (2011).
- Taylor, S. S. *et al.* Structural framework for the protein kinase family. *Annu Rev Cell Biol* **8**, 429–462 (1992).
- Bos, J. L. Epac: A new cAMP target and new avenues in cAMP research. *Nat. Rev. Mol. Cell Biol.* **4**(9), 733–738 (2003).
- Zaccolo, M. & Pozzan, T. cAMP and Ca²⁺ interplay: A matter of oscillation patterns. *Trends Neurosci.* **26**(2), 53–55 (2003).
- Brand, T. The Popeye domain-containing gene family. *Cell Biochem. Biophys.* **43**(1), 95–103 (2005).
- Brisson, G. R., Malaisse-Lagae, F. & Malaisse, W. J. The stimulus-secretion coupling of glucose-induced insulin release. VII. A proposed site of action for adenosine-3',5'-cyclic monophosphate. *J. Clin. Investig.* **51**(2), 232–241 (1972).
- Rous, S. Effect of dibutyl cAMP on the enzymes of fatty acid synthesis and of glycogen metabolism. *FEBS Lett.* **12**(1), 45–48 (1970).
- Geelen, M. J. & Vaartjes, W. J. Levels of cyclic 3'-5'-adenosine monophosphate (cAMP) in maintenance cultures of rat hepatocytes in response to insulin and glucagon. *Lipids* **12**(7), 577–580 (1977).
- Stork, P. J. & Schmitt, J. M. Crosstalk between cAMP and MAP kinase signaling in the regulation of cell proliferation. *Trends Cell Biol.* **12**(6), 258–266 (2002).
- Weisinger, E. M. *et al.* Inhibition of the Raf-1 kinase by cyclic AMP agonists causes apoptosis of v-abl-transformed cells. *Mol. Cell Biol.* **17**(6), 3229–3241 (1997).
- Suen, D. F., Norris, K. L. & Youle, R. J. Mitochondrial dynamics and apoptosis. *Genes Dev.* **22**(12), 1577–1590 (2008).
- Yamamoto, K. K., Gonzalez, G. A., Biggs, W. H. 3rd. & Montminy, M. R. Phosphorylation-induced binding and transcriptional efficacy of nuclear factor CREB. *Nature* **334**(6182), 494–498 (1988).
- Chen, L. *et al.* Mutation of an A-kinase-anchoring protein causes long-QT syndrome. *Proc. Natl. Acad. Sci. USA* **104**(52), 20990–20995 (2007).
- Marx, S. O. *et al.* Requirement of a macromolecular signaling complex for beta adrenergic receptor modulation of the KCNQ1-KCNE1 potassium channel. *Science* **295**(5554), 496–499 (2002).
- Bernstein, H. G. *et al.* Increased density of AKAP5-expressing neurons in the anterior cingulate cortex of subjects with bipolar disorder. *J. Psychiatr. Res.* **47**(6), 699–705 (2013).
- Reissner, K. J. Proteomic analyses of PKA and AKAP signaling in cocaine addiction. *Neuropsychopharmacology* **38**(1), 251–252 (2013).
- Richter, S. *et al.* A potential role for a genetic variation of AKAP5 in human aggression and anger control. *Front. Hum. Neurosci.* **5**, 175 (2011).
- Millar, J. K. *et al.* DISC1 and PDE4B are interacting genetic factors in schizophrenia that regulate cAMP signaling. *Science* **310**(5751), 1187–1191 (2005).
- Marquette, A. *et al.* ERK and PDE4 cooperate to induce RAF isoform switching in melanoma. *Nat. Struct. Mol. Biol.* **18**(5), 584–591 (2011).
- Landis, C. A. *et al.* GTPase inhibiting mutations activate the alpha chain of Gs and stimulate adenylyl cyclase in human pituitary tumours. *Nature* **340**(6236), 692–696 (1989).
- Hinke, S. A. *et al.* Anchored phosphatases modulate glucose homeostasis. *Embo J.* **31**(20), 3991–4004 (2012).
- Zhang, C. L. *et al.* The cAMP sensor Epac2 is a direct target of antidiabetic sulfonylurea drugs. *Science* **325**(5940), 607–610 (2009).
- Czyzyk, T. A., Sikorski, M. A., Yang, L. & McKnight, G. S. Disruption of the RIIbeta subunit of PKA reverses the obesity syndrome of Agouti lethal yellow mice. *Proc. Natl. Acad. Sci. USA* **105**(1), 276–281 (2008).
- Calvin, H. I. *et al.* Induction of cortical cataracts in cultured mouse lenses with H-89, an inhibitor of protein kinase A. *Curr. Eye Res.* **27**(5), 269–278 (2003).
- Chochung, Y. Differentiation therapy of cancer targeting the ri-alpha regulatory subunit of cAMP-dependent protein-kinase (review). *Int. J. Oncol.* **3**(2), 141–148 (1993).
- Sapio, L. *et al.* Targeting protein kinase A in cancer therapy: An update. *Excli J.* **13**, 843–855 (2014).
- Miller, R. A. *et al.* Biguanides suppress hepatic glucagon signalling by decreasing production of cyclic AMP. *Nature* **494**(7436), 256–260 (2013).
- Torres, V. E. & Harris, P. C. Strategies targeting cAMP signaling in the treatment of polycystic kidney disease. *J. Am. Soc. Nephrol.* **25**(1), 18–32 (2014).
- Wouters, E. F. *et al.* Effect of the phosphodiesterase 4 inhibitor roflumilast on glucose metabolism in patients with treatment-naive, newly diagnosed type 2 diabetes mellitus. *J. Clin. Endocrinol. Metab.* **97**(9), E1720–E1725 (2012).
- Toya, Y., Schwencke, C. & Ishikawa, Y. Forskolin derivatives with increased selectivity for cardiac adenylyl cyclase. *J. Mol. Cell Cardiol.* **30**(1), 97–108 (1998).
- Beghè, B., Rabe, K. F. & Fabbri, L. M. Phosphodiesterase-4 inhibitor therapy for lung diseases. *Am. J. Respir. Crit. Care Med.* **188**(3), 271–278 (2013).
- Mullhall, A. M. *et al.* Phosphodiesterase 4 inhibitors for the treatment of chronic obstructive pulmonary disease: A review of current and developing drugs. *Expert Opin. Investig. Drugs* **24**(12), 1597–1611 (2015).
- England, C. G., Ehlerding, E. B. & Cai, W. NanoLuc: A small luciferase is brightening up the field of bioluminescence. *Bioconjug. Chem.* **27**(5), 1175–1187 (2016).
- Fan, F. & Wood, K. V. Bioluminescent assays for high-throughput screening. *Assay Drug Dev. Technol.* **5**(1), 127–136 (2007).
- Dixon, A. S. *et al.* NanoLuc complementation reporter optimized for accurate measurement of protein interactions in cells. *ACS Chem. Biol.* **11**(2), 400–408 (2016).
- Zhang, J. H., Chung, T. D. & Oldenburg, K. R. A simple statistical parameter for use in evaluation and validation of high throughput screening assays. *J. Biomol. Screen.* **4**(2), 67–73 (1999).
- Hwang, B. B., Engel, L., Goueli, S. A. & Zegzouti, H. A homogeneous bioluminescent immunoassay to probe cellular signaling pathway regulation. *Commun. Biol.* **3**, 8 (2020).
- Conti, M. *et al.* Cyclic AMP-specific PDE4 phosphodiesterases as critical components of cyclic AMP signaling. *J. Biol. Chem.* **278**(8), 5493–5496 (2003).
- Bobin, P. *et al.* Cyclic nucleotide phosphodiesterases in heart and vessels: A therapeutic perspective. *Arch. Cardiovasc. Dis.* **109**(6–7), 431–443 (2016).

43. Phillips, J. E. Inhaled phosphodiesterase 4 (PDE4) inhibitors for inflammatory respiratory diseases. *Front. Pharmacol.* **11**, 259 (2020).
44. Sriram, K. & Insel, P. A. G protein-coupled receptors as targets for approved drugs: How many targets and how many drugs?. *Mol. Pharmacol.* **93**(4), 251–258 (2018).

Author contributions

S.G. conceived the idea and proposed the experimental design. M.L., H.W. designed the tracers used in the studies. D.M., N.M., K.H., T.U. carried out experimental work and data analysis. All authors were involved in writing and editing the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

Supplementary Information The online version contains supplementary material available at <https://doi.org/10.1038/s41598-024-55038-0>.

Correspondence and requests for materials should be addressed to S.A.G.

Reprints and permissions information is available at www.nature.com/reprints.

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Open Access This article is licensed under a Creative Commons Attribution 4.0 International License, which permits use, sharing, adaptation, distribution and reproduction in any medium or format, as long as you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons licence, and indicate if changes were made. The images or other third party material in this article are included in the article's Creative Commons licence, unless indicated otherwise in a credit line to the material. If material is not included in the article's Creative Commons licence and your intended use is not permitted by statutory regulation or exceeds the permitted use, you will need to obtain permission directly from the copyright holder. To view a copy of this licence, visit <http://creativecommons.org/licenses/by/4.0/>.

© The Author(s) 2024