opnMe by Boehringer Ingelheim

opn2EXPERTS – Illuminating the GPCRome

The opnMe GPCR Route 66+ project: Which of your fluorescent ligands can shed light on the selectivity of GPCR-targeting compounds to support faster drug discovery efforts?

Answers to this question including a proposal for collaboration can only be considered if they arrive no later than September 27, 2023, 11:59 pm PST.

Boehringer Ingelheim

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What is the context of the problem that we would like to solve?

Cellular signaling pathways involving G protein-coupled receptors (GPCRs) regulate a wide range of physiological processes, such as vision, olfaction, hormone regulation, and neurotransmission, among others[1,2]. Consequently, GPCRs can play central roles in several pathologies, which make them prime targets of several successful medicines and many ongoing drug discovery efforts. Owing to their multiple functions, GPCRs can also be the source of drug adverse effects, such as emesis, sedation, and weight gain. Thus, by understanding GPCR selectivity of drug candidates, we can improve their therapeutic index that is a quantitative measurement of relative compound safety. With safety liabilities being the main cause of attrition in early drug development, the identification of potentially unsafe drugs in early phases of the discovery process can help to reduce the overall costs and timelines. To this end, fast and resource-effective methodologies to detect unselective GPCR binding can streamline the selection of safe candidates.

Currently, there are different assay systems commercially available that can be used to investigate GPCR modulation. Although assays such as beta-arrestin recruitment, calcium influx, receptor internalization, and second messenger production are commonly used in GPCR small molecule research, they primarily monitor the GPCR's function. However, these assays do not provide sufficient data resolution to determine a clear mechanism of action regarding the binding and kinetics of GPCR drugs. Therefore, binding characteristics such as kinetic selectivity – a parameter of increasing importance in pharmacometrics - cannot be thoroughly investigated.

To enable the scientific community to study the molecular mechanism of ligand – GPCRs interaction, scientists from the High Throughput Biology department at Boehringer Ingelheim have launched the **opnMe GPCR Route 66+ project**. The goal is to setup a GPCR selectivity binding assay panel based on the TR-FRET[3] technology. This should enable large-scale profiling of the binding affinities and kinetics of test compounds for a variety of receptors. As a tracer displacement technology, TR-FRET may enable research on tracer based binding kinetics, molecular mechanism of action studies and determination of orthosteric or allosteric binding sites based on the tracer properties. To facilitate the process, we prepared 66 suitable GPCR constructs that are ready for human cell culture expression. To fulfill our mission of providing the scientific community with a high-quality, broad GPCR selectivity panel, we require your assistance in obtaining suitable fluorescent probes, also known as tracers, which are currently limited.

With the opnMe GPCR Route 66+ call, we are reaching out to the GPCR community to contribute novel tracers to expand the breadth of the panel to the highest possible number of targets. To this end, we offer our commitment to establish and validate assays using these tracers at our own costs, and to share the resulting protocol including documentation with the probe owners first, and with the scientific community later as part of an open access publication co-authored by all contributors of the final tracer set. In addition, we provide contributors of the final tracer set with the opportunity to propose candidate molecules for

inclusion in the tests on the resulting broad GPCR panel to determine their affinities and kinetic binding properties.

This open science collaboration should rapidly enable scientists across the globe to perform comprehensive GPCR selectivity profiling for their compounds of interest, which will ultimately benefit the development of safer and more effective drugs.

What potential solutions could be in scope?

The scope of your contributions includes any fluorescently labelled ligand that binds ortho- or allosterically to one or more of the 66 GPCRs of interest for this project. Find an overview of the list and a depiction (figure 1) below and the complete list as part of the appendix (also available as a downloadable Excel-File on opnMe):

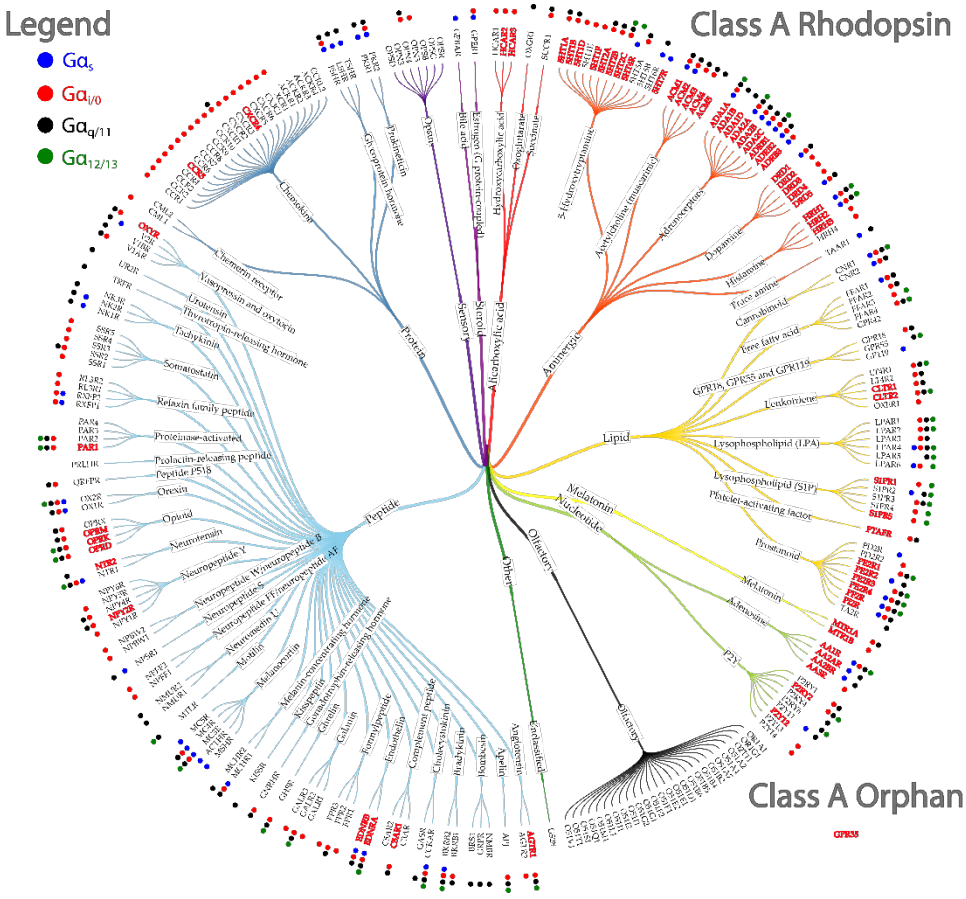

Figure 1 : Overview of the 66 GPCRs of interest in this call. GPCRs of interest are written in red. The signaling according to GPCRdb is shown as presented in the figure legend. Picture modified from4.

GPCR subfamily/ligand	GPCR of interest
5-Hydroxytryptamine	5HT1A, 5HT1B, 5HT1D, 5HT1F, 5HT2A, 5HT2B, 5HT2C, 5HT4R, 5HT7R
Acetylcholine	ACM1, ACM2, ACM3, ACM4, ACM5
Adenosine	AA1R, AA2AR, AA2BR, AA3R
Adrenaline	ADA1A, ADA1B, ADA1D, ADA2A, ADA2B, ADA2C, ADRB1, ADRB2, ADRB3
Chemokine	CCR5, CXCR4
Dopamine	DRD1, DRD2, DRD3, DRD4, DRD5
Endothelin	EDNRA, EDNRB
Histamine	HRH1, HRH2, HRH3
Hydroxycarboxylic acid	HCAR2, HCAR3
Leukotriene	CLTR1, CLTR2
Melatonine	MTR1A, MTR1B
Opioid	OPRD, OPRK, OPRM
P2Y	P2RY2, P2Y12
Prostaglandin	PE2R1, PE2R2, PE2R3, PE2R4, PI2R, PF2R
S1P	S1PR1, S1PR5
Other	AGTR1, C5AR1, NPY2R, NTR2, OXYR, PTAFR, PAR1, GPR35

In addition, the profile of these ligands should fulfill the following criteria:

- They can be fragments, and/or small molecules, and/or peptides, and/or any biologics (antibodies, nanobodies, aptamers, etc.) and/or endogenous ligands with affinities (KD) for the considered GPCR better than 300 nM.
- Their fluorescent moieties should be excitable at 490, 548, 587 or 621 nm, and emit at 515 nm or above 650 nm. Alternatively, we accept biotin-adducts of your ligand that can easily be coupled to the desired fluorophores.
- They should be chemically stable and preferably not cytotoxic at test concentrations.
- They can target several GPCRs with similar affinities, their selectivity against other GPCRs is not a key criterion for the selection of the ligand (a broad coverage across sub-families would even be preferred).
- Optional: For planned assay validations, it will be beneficial to receive also unlabeled tracer control(s) as part of your submission.

For all submissions: The chemical structures of all submitted tracer(s) and control(s) **SHOULD NOT** be disclosed in your application. Only information on their molecular weight and spectral properties must be shared as part of this opnMe call.

What potential solutions would be out of scope?

The following ligands will be considered out of scope:

- Binding affinities of the ligand far above 300 nM.
- Fluorescent moieties that don't meet the spectral property criteria mentioned above.
- Chemically unstable and strong cytotoxic molecules (EC_{50} > 10 µM).
- Radioisotope labeled molecules.
- Commercially available molecules.
- Alternative technological solutions like e.g., SPR, DSF and others.

How is this opn2EXPERTS question structured?

This opn2EXPERTS call is structured in two main phases and with the current call on opnMe.com, the first phase is initiated. For detailed information please click on the respective chapters below.

The table below provides an overview on the different phases of this call. The first Phase (Phase 1a) has been initiated and continues until September 27, 2023, 11:59 pm PST.

Phase	PHASE 1A	PHASE 1B	PHASE 2A	PHASE 2B	PHASE 2C
Timeline	June 11, 2023 – September 27, 2023	September 28, 2023 – March 29, 2024	April 1, 2024 – April 30, 2024	May 2, 2024 – March 31, 2025	April 1, 2025 – December 31, 2025
Our action	This call is open for proposals.	Characterization of your tracers: on-target K_D on-target k_{on}/k_{off} Unspecific cytotoxicity EC_{50} Control Compound EC_{50}	April 1, 2024: Contacting the winners.	Generation of GPCR selectivity profiling data on selected GPCR drugs.	Publication
Your action	You send in GPCR tracers meeting criteria using submission form.		You provide more of your tracer if required.		For selected tracer molecules: Disclose your structures to us and give permission to include them in the final publication due December 31, 2025. Contribute to the publication and approve final publication draft.
IP	IP remains with you. Don't disclose structures.	IP remains with you. Don't disclose structures.	Your window to decide to patent your IP.	Your window to decide to patent your IP.	IP strategy must be completed. Tracer structure should be disclosed for

					inclusion in the final publication.
Milestone	All submissions comprise the final set of GPCR tracer candidates.	Assay development complete.	March 29, 2024: Selection of winners.	GPCR Dataset complete.	Publication complete.
Your Benefits	Get reimbursed for shipment (200 euros).	Get characterization data for your tracer molecule including an optimized assay protocol.		Get an aliquot of the TR-FRET Plasmid used for your tracer on demand. Selected tracer molecules win 2,000 euros. Submit small molecule test candidates to be considered for GPCR profiling. Get pre-publication access to the GPCR dataset.	Co-authorship for selected final tracer.

Phase 1 (June 12, 2023 – March 29, 2024) - Reagents (Phase 1a) and Assay Development (Phase 1b):

How to participate - Your action:

Until the end of the submission time of this first phase (Phase 1a), we invite all experts in the field of GPCRs to share 1 mg of their fluorescent tracer(s) with us for the above-described receptors using the submission document provided with this call. To facilitate the pre-selection process, we highly recommend including data showing that your probes fulfill the above-mentioned criteria. The submission document will also require your signature as it serves as a material transfer agreement that defines and secures your intellectual property contribution that ONLY provides Boehringer Ingelheim with the license to develop and perform an assay based on your shared fluorescent probe (tracer). In fact, we kindly ask you not to disclose your fluorescent probe's structures or SMILES codes at this point. Submissions are accepted through September 27th, 2023, 11:59 pm PST.

Our commitment:

On our end, we are committed to characterize the equilibrium binding and kinetic properties and the selectivities of all submitted tracers that meet the criteria listed above within six months (Phase 1b), and to share with each applicant all results that Boehringer Ingelheim generated, including a license to use these data freely and the right to publish after finalization of phase 2.

Your Benefits:

As a participant to this opn2EXPERTS call, you will have the chance to outline and share your expertise, skill set, and prior successes in the field with a top 20 pharmaceutical company. For phase 1, you will receive 200 euros as a reimbursement of your shipping costs for the fluorescent tracer(s) delivery for a maximum of one delivery. We kindly ask you to refrain from sending individual shipments for individual tracers; rather we encourage you to combine the shipment. The assay development at Boehringer Ingelheim including the other reagents needed will be covered by Boehringer Ingelheim. You will receive all data that Boehringer Ingelheim will generate, and the potential prospect for a publication on this assay development. Overall, it opens the opportunity to work on a scientific topic in the field of GPCR and accelerate the drug development of potential compounds targeting essential GPCRs. All submitted IP will continue to stay with you and you are free to use it for other purposes.

Milestone:

Based on the data generated for all submitted tracer molecules, we will decide on a final tracer set that serves as the reagent collection for the future GPCR selectivity panel. As decision criteria for the selection, we apply our "in scope" criteria to move to Phase 2. To summarize, your tracer

- was detectable using the TR-FRET assay set-up,

- shows sufficient potency,
- shows favorable (fast) kinetic binding properties,
- shows no cytotoxicity at test concentrations, and
- was used to determine a control compound EC_{50} (for example using an unlabeled version of your molecule or a known drug) [optional]

In addition, the number of tracer candidates submitted per person and GPCR target must be limited to 10 for logistical reasons. In case of similar entries from different participants that meet all quality criteria, we reserve the right to select the final molecule.

Phase 2 (April 1, 2024 – December 31, 2025) - Winner selection (Phase 2a), GPCR selectivity assessment (Phase 2b) and publication (Phase 2c):

Our commitment:

As part of Phase 2, we plan to characterize and study the selectivity of a broader small molecule GPCR drug set (> 300 drugs) both looking at equilibrium and binding kinetic properties where feasible based on the tracers submitted. After thorough analysis we plan to publish the results in a co-authored publication by December 31, 2025.

Depending on the outcome of in-depths characterization analyses conducted on our end as part of phase 1, we might be interested in obtaining an additional sample of your submitted tracer(s). Should your tracer(s) be affected, we will contact you at that time for next steps.

Your Benefits:

You will benefit from receiving a 2,000 euros award per accepted and validated fluorescent probe that will serve as a non-exclusive license fee. Also, you will receive a detailed protocol describing the assay conditions including detailed validation data. You may have the opportunity to receive the plasmid-DNA to set up the assay in your own laboratory. In addition, we offer you the possibility to co-author a broader scope scientific publication describing our overall efforts to improve early drug safety screening in a high impact factor journal upon conclusion of this call. To be eligible for co-authorship you should be willing to disclose your tracer structure in the publication.

Furthermore, you will have the opportunity to nominate a maximum of five compound tools for validation studies and gain pre-publication access to the data of the broader GPCR panel generated. Your contribution will be valued in the community and offers you the possibility of high visibility as well as a network opportunity and link with likeminded scientists.

Key criteria for the best answer:

Your tracer(s) will be selected from all submissions based on the criteria listed in Phase 1. We reserve the right to select the winner based on the tracer properties including potency, binding kinetic properties, cytotoxicity, and the ability to be used for a successful EC_{50}

determination. In case of multiple potential tracer submissions for one of the 66 GPCR assays, we may select the tracer that covers more than one GPCR to minimize reagents needed for the final panel.

Anticipated Project Phases or Project Plan

Phase 1	Please complete your submission for Phase 1 by September 27, 2023, 11:59 pm PST. Our review of all proposals will be completed by March 29, 2024.
Phase 2	Winning submissions for Phase 2 will be informed in w/c April 1, 2024, about next steps.

Submitting a collaboration proposal as part of Phase 1

- Check the outline of the opnMe GPCR Route 66+ call "Illuminating the GPCRome" on opnMe.
- Alternatively, you may click the "Get Submission Template" banner to access the material transfer template.
- Follow the instructions to upload your submission document (requires login or registration).
- The upload allows you to attach additional application files if desired.
- You will be able to access your final submitted proposal in your personal dashboard and follow its review status.
- Please also visit the specific FAQ section on opnMe.com to learn more about this program.
- Please use our answer submission template to provide non-confidential* information about your submitted tracer(s), such as your tracer's MW, concentration, mass, and spectral properties as well as the expected affinity to the GPCRs proposed (available for download on the following site). Please note that the submission document also serves as a material transfer document that will require your signature to confirm that IP of the submitted tracer(s) remain with you.

Chemical structures **SHOULD NOT be disclosed in your initial application.*

References

1. Hauser AS, Attwood MM, Rask-Andersen M, Schiöth HB, Gloriam DE. Trends in GPCR drug discovery: new agents, targets and indications. *Nat Rev Drug Discov.* **2017**;16(12):829-842. doi: 10.1038/nrd.2017.178, PubMed
2. Sriram K, Insel PA. G Protein-Coupled Receptors as Targets for Approved Drugs: How Many Targets and How Many Drugs? *Mol Pharmacol.* **2018**;93(4):251-258. doi: 10.1124/mol.117.111062, PubMed
3. Zwier JM, Roux T, Cottet M, Durroux T, Douzon S, Bdioui S, Gregor N, Bourrier E, Oueslati N, Nicolas L, Tinel N. A fluorescent ligand-binding alternative using Tag-lite® technology. *Journal of biomolecular screening*. **2010**;15(10):1248-59. doi: 10.1177/1087057110384611, PubMed
4. The G protein database, GproteinDb. Pandy-Szekeres G, Esguerra M, Hauser AS, Caroli J, Munk C, Pilger S, Keseru GM, Kooistra AJ, Gloriam DE, *Nucleic acids research*, **2022**, 50:D518-D525. doi: 10.1093/nar/gkab852 , PubMed

Appendix

Tabular overview of all 66 GPCRs of this opnMe call:

Uniprot Name	Uniprot ID	Name	Ligand
5HT1A_HUMAN	P08908	Serotonin 5HT1A	5-Hydroxytryptamine
5HT1B_HUMAN	P28222	Serotonin 5HT1B	5-Hydroxytryptamine
5HT1D_HUMAN	P28221	Serotonin 5HT1D	5-Hydroxytryptamine
5HT1F_HUMAN	P30939	Serotonin 5HT1F	5-Hydroxytryptamine
5HT2A_HUMAN	P28223	Serotonin 5HT2A	5-Hydroxytryptamine
5HT2B_HUMAN	P41595	Serotonin 5HT2B	5-Hydroxytryptamine
5HT2C_HUMAN	P28335	Serotonin 5HT2C	5-Hydroxytryptamine
5HT4R_HUMAN	Q13639	Serotonin 5HT4	5-Hydroxytryptamine
5HT7R_HUMAN	P34969	Serotonin 5HT7	5-Hydroxytryptamine
ACM1_HUMAN	P11229	Muscarinic acetylcholine receptor M1	Acetylcholine
ACM2_HUMAN	P08172	Muscarinic acetylcholine receptor M2	Acetylcholine
ACM3_HUMAN	P20309	Muscarinic acetylcholine receptor M3	Acetylcholine
ACM4_HUMAN	P08173	Muscarinic acetylcholine receptor M4	Acetylcholine
ACM5_HUMAN	P08912	Muscarinic acetylcholine receptor M5	Acetylcholine
AA1R_HUMAN	P30542	Adenosine A1 receptor	Adenosine

Uniprot Name	Uniprot ID	Name	Ligand
AA2AR_HUMAN	P29274	Adenosine A2A receptor	Adenosine
AA2BR_HUMAN	P29275	Adenosine A2B receptor	Adenosine
AA3R_HUMAN	P0DMS8	Adenosine A3 receptor	Adenosine
ADA1A_HUMAN	P35348	Alpha-1A adrenergic receptor	Adrenaline
ADA1B_HUMAN	P35368	Alpha-1B adrenergic receptor	Adrenaline
ADA1D_HUMAN	P25100	Alpha-1D adrenergic receptor	Adrenaline
ADA2A_HUMAN	P08913	Alpha-2A adrenergic receptor	Adrenaline
ADA2B_HUMAN	P18089	Alpha-2B adrenergic receptor	Adrenaline
ADA2C_HUMAN	P18825	Alpha-2C adrenergic receptor	Adrenaline
ADRB1_HUMAN	P08588	Beta-1 adrenergic receptor	Adrenaline
ADRB2_HUMAN	P07550	Beta-2 adrenergic receptor	Adrenaline
ADRB3_HUMAN	P13945	Beta-3 adrenergic receptor	Adrenaline
AGTR1_HUMAN	P30556	Angiotensin receptor AT1 receptor	Angiotensin
CCR5_HUMAN	P51681	C-C chemokine receptor type 5	Chemokine

Uniprot Name	Uniprot ID	Name	Ligand
CXCR4_HUMAN	P61073	C-X-C chemokine receptor type 4	Chemokine
C5AR1_HUMAN	P21730	C5a Receptor	Complement peptide
DRD1_HUMAN	P21728	Dopamine D1 receptor	Dopamine
DRD2_HUMAN	P14416	Dopamine D2 receptor	Dopamine
DRD3_HUMAN	P35462	Dopamine D3 receptor	Dopamine
DRD4_HUMAN	P21917	Dopamine D4 receptor	Dopamine
DRD5_HUMAN	P21918	Dopamine D5 receptor	Dopamine
EDNRA_HUMAN	P25101	Endothelin A receptor	Endothelin
EDNRB_HUMAN	P24530	Endothelin B receptor	Endothelin
HRH1_HUMAN	P35367	Histamine H1 receptor	Histamine
HRH2_HUMAN	P25021	Histamine H2 receptor	Histamine
HRH3_HUMAN	Q9Y5N1	Histamine H3 receptor	Histamine
HCAR2_HUMAN	Q8TDS4	Hydroxycarboxylic acid receptor 2	Hydroxycarboxylic acid
HCAR3_HUMAN	P49019	Hydroxycarboxylic acid receptor 3	Hydroxycarboxylic acid
CLTR1_HUMAN	Q9Y271	Cysteinyl leukotriene receptor 1	Leukotriene
CLTR2_HUMAN	Q9NS75	Cysteinyl leukotriene receptor 2	Leukotriene
MTR1A_HUMAN	P48039	Melatonin receptor type 1A	Melatonin

Uniprot Name	Uniprot ID	Name	Ligand
MTR1B_HUMAN	P49286	Melatonin receptor type 1B	Melatonin
NPY2R_HUMAN	P49146	Neuropeptide Y receptor type 2	Neuropeptide Y
NTR2_HUMAN	O95665	Neurotensin receptor type 2	Neurotensin
OPRD_HUMAN	P41143	Opioid receptors δ receptor	Opioid
OPRK_HUMAN	P41145	Opioid receptors κ receptor	Opioid
OPRM_HUMAN	P35372	Mu-type opioid receptor	Opioid
OXYR_HUMAN	P30559	Oxytocin receptor	Oxytocin
P2RY2_HUMAN	P41231	P2Y purinoceptor 2	P2Y
P2Y12_HUMAN	Q9H244	P2Y purinoceptor 12	P2Y
PTAFR_HUMAN	P25105	Platelet-activating factor receptor	Platelet-activating factor
PE2R1_HUMAN	P34995	Prostaglandin E2 receptor EP1 subtype	Prostaglandin
PE2R2_HUMAN	P43116	Prostaglandin E2 receptor EP2 subtype	Prostaglandin
PE2R3_HUMAN	P43115	Prostaglandin E2 receptor EP3 subtype	Prostaglandin
PE2R4_HUMAN	P35408	Prostaglandin E2 receptor EP4 subtype	Prostaglandin
PI2R_HUMAN	P43119	prostaglandin I2 receptor	Prostaglandin
PF2R_HUMAN	P43088	Prostaglandin F2-alpha receptor	Prostaglandin

Uniprot Name	Uniprot ID	Name	Ligand
PAR1_HUMAN	P25116	Proteinase-activated receptors	Proteinase activated
S1PR1_HUMAN	P21453	Sphingosine 1-phosphate receptor 1	S1P
S1PR5_HUMAN	Q9H228	Sphingosine 1-phosphate Receptor 5	S1P
GPR35_HUMAN	Q9HC97	Kynurenic acid receptor	Kynurenic Acid