DALRIADA
TURN-KEY™
DRUG DISCOVERY

Biased Signaling as a Catalyst for Precision GPCR Drug Discovery

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Dalriada Drug Discovery

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Summary
The white paper explores the immense therapeutic potential of G protein-coupled receptors (GPCRs), the largest family of membrane proteins in the human genome, comprising more than 800 members. GPCRs regulate diverse physiological processes, including sensory perception, immune responses, and cellular signaling. A key focus of this paper is GPCR drug discovery biased signaling, an emerging paradigm where ligands selectively stabilize specific receptor conformations to activate distinct pathways. This approach enables researchers to decouple therapeutic efficacy from adverse effects by targeting beneficial pathways while avoiding deleterious ones. New innovations are discussed that reshape GPCR-targeted drug discovery by unlocking previously inaccessible receptor targets and driving next-generation therapeutics with enhanced efficacy and safety profiles.

Advances in GPCR Drug Discovery

GPCRs are among the most successful drug targets in modern medicine, yet many remain underexploited due to the complexity of their signaling pathways and limitations in traditional screening methods (1). Recent breakthroughs in structural biology, particularly through cryo-electron microscopy (cryo-EM) and X-ray crystallography—have transformed the field (2). These techniques have enabled high-resolution visualization of GPCRs in their active, inactive, and intermediate states, revealing new ligand-binding pockets and providing insights into biased signaling. This understanding is critical for designing selective modulators that enhance therapeutic benefit while minimizing side effects (3).
Complementing these structural advances, AI-powered tools such as AlphaFold2 accelerate the prediction of receptor conformations and dynamics (4). These technologies facilitate structure-based drug design by modeling how potential drugs interact with GPCRs at the molecular level.

By integrating structural data with machine learning, researchers can identify novel druggable targets and develop compounds with pathway-specific activity. Together, these innovations are redefining how GPCR-targeted therapies are discovered and optimized, offering new hope for tackling previously intractable diseases. Importantly, these computational advances must be complemented by wet-lab experimentation, which validates predictions and translates them into clinically relevant therapies.

GPCR Biased Signaling

Biased signaling is an emerging paradigm in GPCR research where ligands selectively stabilize specific receptor conformations to activate distinct intracellular pathways (5). Rather than uniformly triggering all downstream responses, certain ligands can preferentially activate either G protein or arrestin-mediated signaling, or even particular subtypes within these pathways. This molecular selectivity enables researchers and drug developers to decouple therapeutic efficacy from adverse effects by targeting beneficial pathways linked to

desired outcomes, while minimizing activation of signaling routes associated with deleterious side effects. Ultimately, biased signaling offers a sophisticated approach to designing next-generation therapeutics with improved safety and effectiveness.

Oliceridine is a prime example of a G protein-biased agonist developed to target the μ-opioid receptor (μ-OR). Traditional opioids, like morphine, activate both G protein and β-arrestin pathways upon binding to μ-OR (6). While G protein signaling is primarily responsible for analgesic effects, β-arrestin recruitment is linked to many of the adverse effects of opioids, such as respiratory depression, constipation, and tolerance . Oliceridine preferentially activates G protein signaling over β-arrestin pathways. This selective signaling is achieved by the ligand's unique structure, which stabilizes receptor conformations favoring G protein coupling.

Additionally, psychedelic compounds, such as LSD and psilocybin, exert their effects primarily through the 5HT2A serotonin receptor. However, the therapeutic benefits (e.g., antidepressant or anxiolytic effects) and the hallucinogenic side effects are thought to be mediated by distinct intracellular signaling pathways (7). Biased ligands for the 5HT2A receptor are designed to preferentially activate signaling cascades (such as G protein or β-arrestin pathways) associated with therapeutic outcomes, while minimizing activation of pathways linked to hallucinations or other adverse psychiatric effects.

Integrated GPCR Drug Discovery Platform

Methods such as the functional assays employed at Dalriada are required to confirm biased signaling and to translate predictive models into actionable insights for drug discovery. For example, β-arrestin recruitment assays directly measure receptor desensitization and internalization, while cAMP and IP-One assays capture G-protein–mediated signaling events. Additional readouts such as pAKT1, pERK1/2, and calcium flux further expand this toolkit, enabling a comprehensive characterization of pathway bias (Figure 1).

Dalriada is licensed by Health Canada to work with Schedule I controlled substances, allowing us to research and develop cannabinoids, opioids, and psychedelics. The Dalriada team has extensive expertise in receptor signaling pathways, employing a suite of advanced functional assays.

Leveraging this experience, we provide state-of-the-art GPCR-biased signaling assays, for example the IP-One assay on 5HT5A receptors, which quantifies inositol monophosphate as a direct readout of Gq/11-coupled receptor activation and is crucial for dissecting serotonergic signaling relevant to neuropsychiatric drug discovery (Figure 2). For cannabinoid research, we utilized CB2 β-arrestin and cAMP assays—the β-arrestin recruitment assay detects receptor desensitization and internalization, while the cAMP assay measures G protein-mediated inhibition of adenylyl cyclase, both essential for evaluating CB2-selective agonist efficacy and safety (Figure 2). Additional assays include pAKT1, pERK1/2, and calcium flux, all performed using cutting-edge technologies like the FLIPR system, enabling comprehensive profiling of compound activity and signaling bias to support the development of safer, more effective therapeutics.

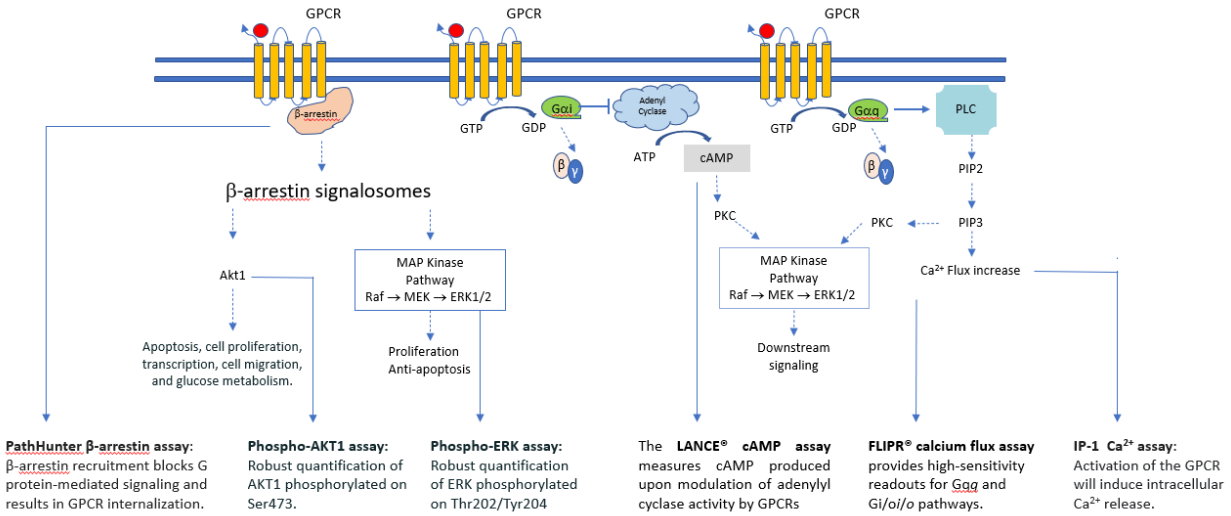

Figure 1. **GPCR Signaling Pathways and Key Output Assays**
This schematic illustrates GPCR signaling dynamics, β-arrestin recruitment, and downstream effector readouts. Upon ligand binding, the GPCR undergoes a conformational change, activating heterotrimeric G proteins (Gα/Gβγ) via GDP/GTP exchange. Gα subunits diverge into subtype-specific pathways: **Gαs** stimulates adenylate cyclase, elevating cAMP and activating protein kinase A which phosphorylates ERK1/2. **Gαq** triggers phospholipase C (PLC), generating inositol trisphosphate (IP3) and diacylglycerol, with IP3 metabolites (e.g., IP-1) serving as biomarkers. β-Arrestin recruitment follows GPCR phosphorylation by kinases terminating G protein signaling and initiating β-arrestin-dependent pathways (e.g., ERK activation). Downstream kinases **pAKT1** (via PI3K-Akt signaling) and **pERK** integrate proliferative and survival signals. The **FLIPR system** monitors real-time calcium flux (via Gq-coupled IP3 receptor activation) or membrane potential changes, enabling high-throughput screening of GPCR and ion channel activity.

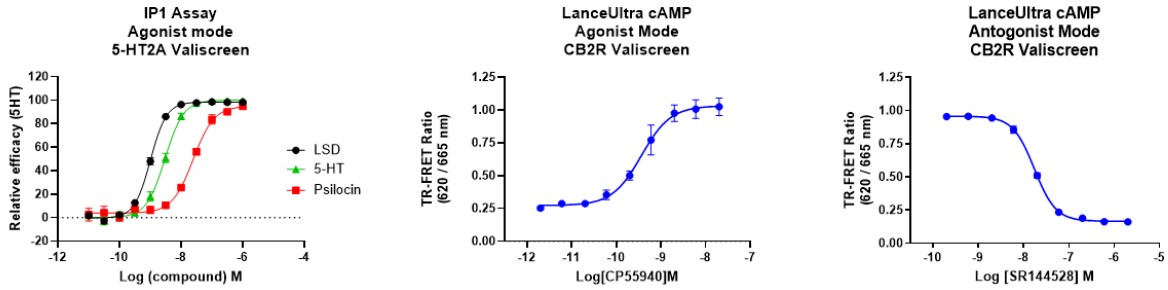

Figure 2. A: Potency Profiles of 5-HT, Psilocin, and DMT in IP-1 Assays Across 5-HT2A. Dose-response curves in CHO-K1 Valiscreen cells expressing 5-HT2A receptors stimulated with reference compounds serotonin (5-HT), psilocin, and DMT. The potency of each compound was determined using EC_{50} values derived from fluorescence resonance energy transfer (FRET)-based homogeneous time-resolved fluorescence (HTRF) assays. B and C. Functional characterization of CP55,940 and SR144528 compounds in cAMP assays using CHO-K1 cells expressing CB2.

References

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3. Manglik A, Kobilka B. The role of protein dynamics in GPCR function: insights from the β2AR and rhodopsin. Curr Opin Cell Biol. 2014;27:136-43.
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6. Gan TJ, Wase L. Oliceridine, a G protein-selective ligand at the μ-opioid receptor, for the management of moderate to severe acute pain. Drugs Today (Barc). 2020;56(4):269-86.
7. Wallach J, Cao AB, Calkins MM, Heim AJ, Lanham JK, Bonniwell EM, et al. Identification of 5-HT. Nat Commun. 2023;14(1):8221.

DALRIADA
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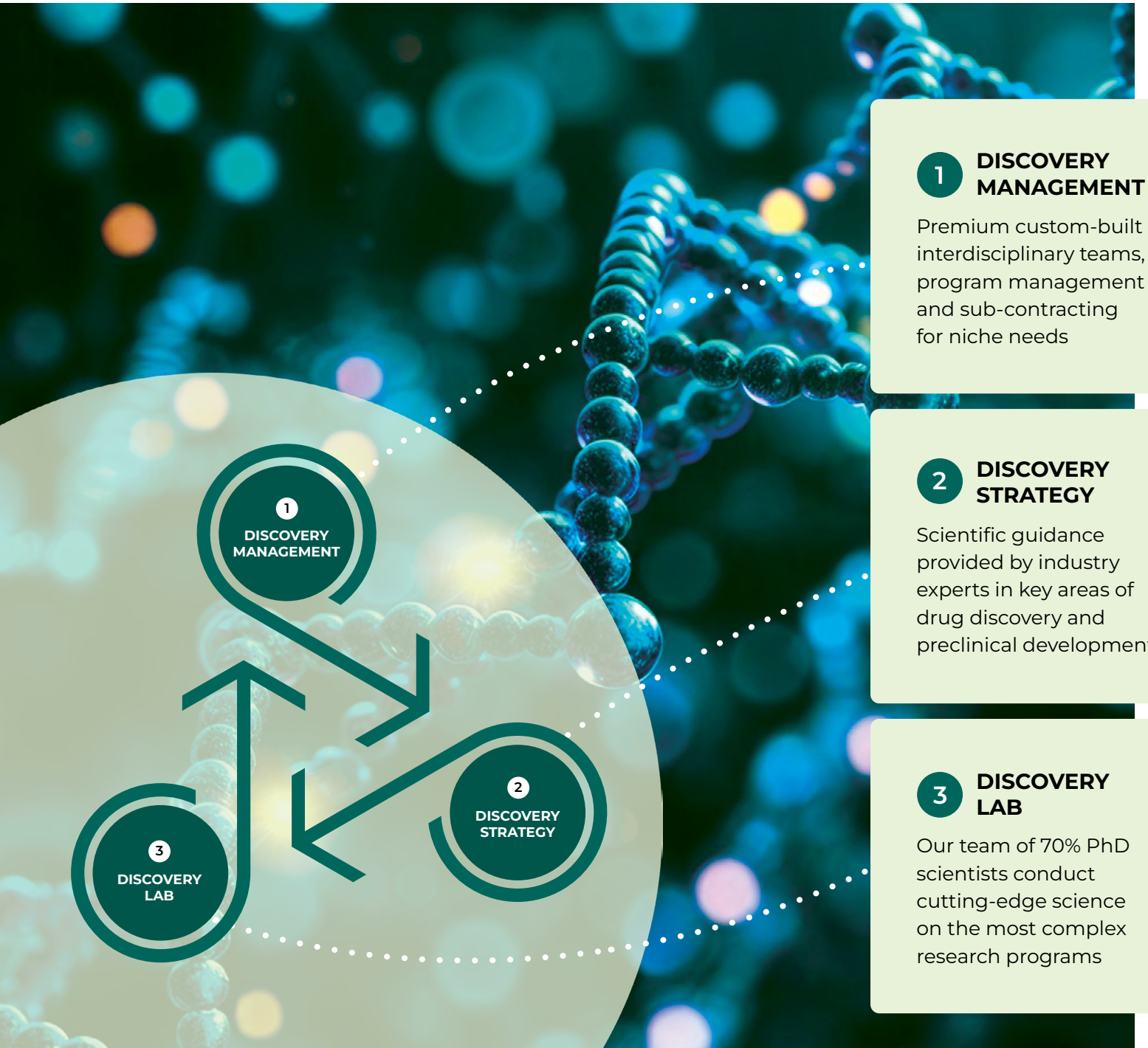
Headquartered in Canada

Supporting drug discovery programs globally

TURN-KEY™ DRUG DISCOVERY



1 DISCOVERY MANAGEMENT

Premium custom-built interdisciplinary teams, program management and sub-contracting for niche needs

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Scientific guidance provided by industry experts in key areas of drug discovery and preclinical development

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DISCOVERY BIOLOGY CAPABILITIES

Protein Sciences

Scalable, high quality protein production

Target identification and construct design

Several protein expression systems: mammalian, Baculovirus, and *E. coli*

Optimization and pilot-scale expression and purification

Large scale protein purification employing affinity and FPLC approaches

Various QC methods including PAGE, aSEC, Thermal Shift Assay, LC/MS/MS

Custom in-house GPCR membrane production

Proteomics

Small molecule Hit-ID and Target-ID

Target site ID selectivity determination

Mechanism of Action (MOA) determination

Determination of protein homeostasis and turnover

Protein-compound binding, covalency and kinetics (k_{inact}/K_I) determination

Protein sequencing, PTM profiling, binding site determination

Biostatistical analysis, network enrichment, hierarchical clustering

Cellular Target Engagement

NanoBRET

HiBiT CETSA (BiTSA)

Jess

Flow Cytometry

Biochemical/Biophysical assays

Custom development of robust and scalable activity/ binding assays

Diverse proteins/enzyme targets (transcription factors, kinases, methyltransferases, PPI...etc.)

Fragment screening

Orthogonal binding techniques including DSF, DSLS, ITC, SPR, BLI, MST, CD, LC-MS/MS

Fluorescence polarization (FP), BRET, FRET, TR-FRET and AlphaLISA assays

Continuous fluorometric assays (AssayQuant etc.)

Kinetics of binding for covalent (k_{inact}/K_I) and non-covalent (K_d) ligands

Cell Viability / IncuCyte Live Cell Imaging

2D/3D cultures

Co-culture assays

Cytotoxicity/proliferation assay

Apoptosis assays (Annexin V/PI, cleaved caspases)

Immune mediating killing of tumor cells

3D Spheroid Killing Assay

Target Modulation and Validation

Loss and gain of function models (shRNA, siRNA, CRISPR, Overexpression)

Immunoprecipitation/ Ubiquitinylation assays

Radiometric assays

Radiometric binding assays (saturation, competition, displacement assays etc.)

Enzyme activity assays (K_m, V_{max}, k_{cat} determination etc.)

Kinetics determinations (on/ off rate, and time course assays)

Radioligand GTPγS functional assays (agonist, antagonists, inverse agonists, EC_{50} Emax determinations)

Licensed from Canadian Nuclear Safety Commission

Signaling and Functional assays

Transcription factor reporter assays

Necroptosis rescue

Inflammasome assay

Calcium flux assay

Secondary messenger (e.g. IP1, cAMP, Ca2+)

Neurotransmitter uptake inhibition assay (SERT/DAT/NET)

Oxidative Stress Assays (Cellular/ Mitochondrial ROS, MitoSOX)

Phosphoprotein measurement (HTRF, AlphaLISA)

Expression profiling

RT-qPCR, RNAseq

Western blot

Quantitative Western (Jess)

ELISA

Luminex MagPix

Flow cytometry

Immunofluorescent and confocal microscopy

HiBiT bioluminescence assay

PROTEIN SCIENCES

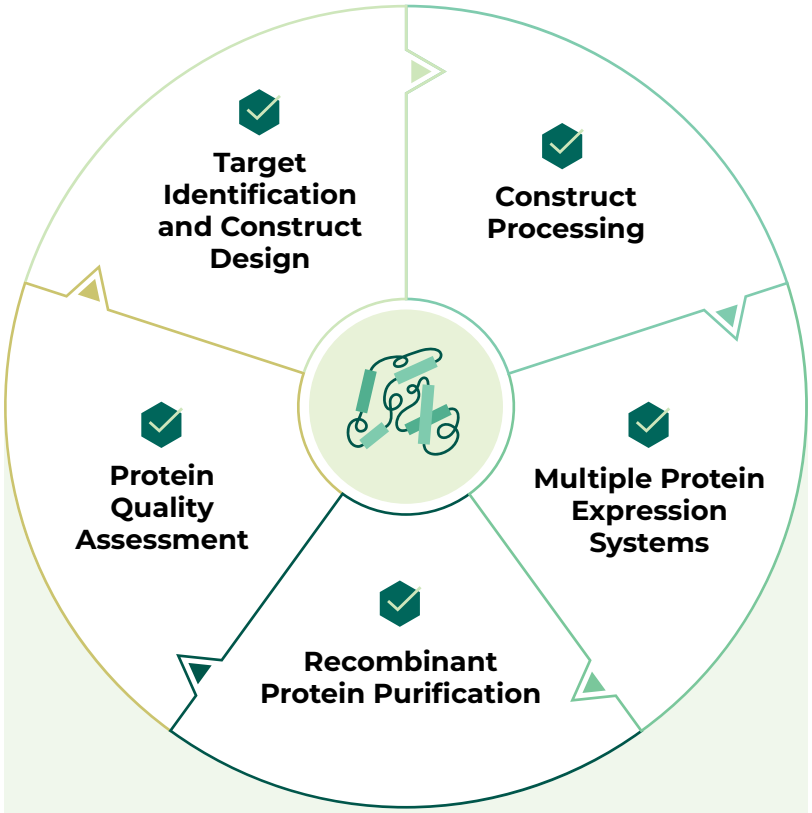
Driving Drug Discovery Through Protein Innovation

Continuous communication to craft a tailored Scope of Work for your project

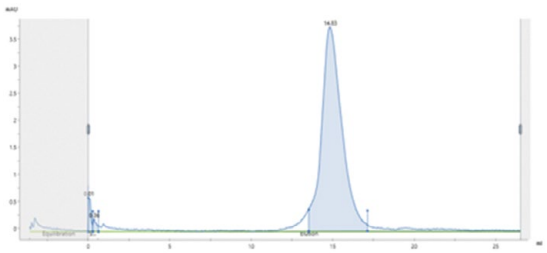
Customized experimental design aligned with your unique requirement

Structured workflows to validate feasibility and reduce project risks

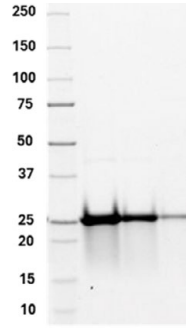
Seamless result delivery with tested quality



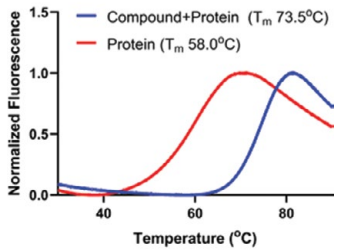
Analytical SEC



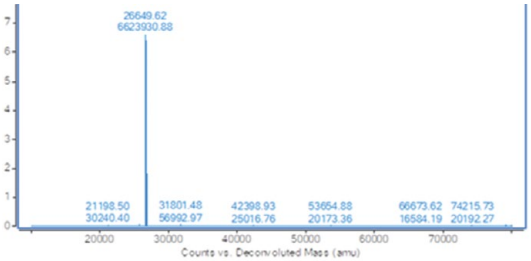
SDS-PAGE



Thermal Shift Assay



Intact Mass Analysis



Getting Started

Construct design
Plasmid DNA amplification
Bacmid and viral stock production
Expression in mammalian, insect and bacterial systems

Production Optimization

Growing conditions, transfection timeline, MOI, time of harvest, optimization for yield and purity

Pilot & Large-Scale Production

Parallel testing of multiple constructs
30-50 L of weekly production capacity

Target Purification

Affinity chromatography on IMAC, FLAG, GST, Avidin resins, Size-Exclusion (SEC), ion-exchange, Heparin and IgG columns

Protein Quality Assessment

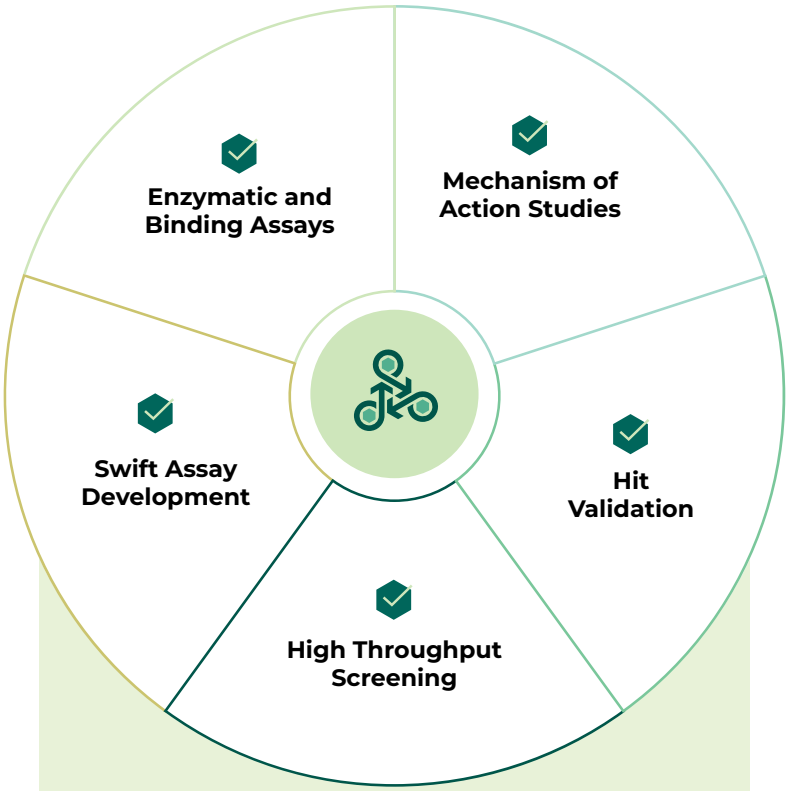
SDS-PAGE, WB, Intact Mass Analysis, protein quantification, analytical SEC, and Thermal Shift Assay

BIOCHEMISTRY AND BIOPHYSICS

Advancing Drug Discovery: Identification and Optimization of Hits

DRIVE YOUR RESEARCH FORWARD WITH OUR COMPREHENSIVE ASSAY SOLUTIONS

FP Assays	Kinetics of binding: covalent (k_{inact}/K_I)
TR FRET	Non-covalent (K_d) ligands
AlphaLISA	
Luminescence	
Continuous Assays	SPR/BLI, ITC
Endpoint Assays	DSF/DSLS
Ternary Complexes	MST

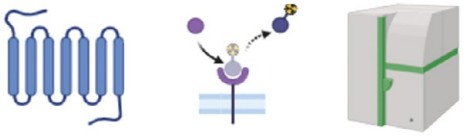


Radioactivity license from the Canadian Nuclear Safety Commission

Radiometric Capabilities

Specialized in developing radioligand binding assays, particularly for membrane-bound targets such as GPCRs and transporters.

GTPγS functional assays to identify agonists and antagonists.




Tecan Spark 10M


MicroCal PEAQ-ITC Automated


LightCycler 480 Instrument II


Stargazer 2 (DSLS)


Formulatrix FAST™


Tecan D300e

PROTEIN MASS SPECTROMETRY

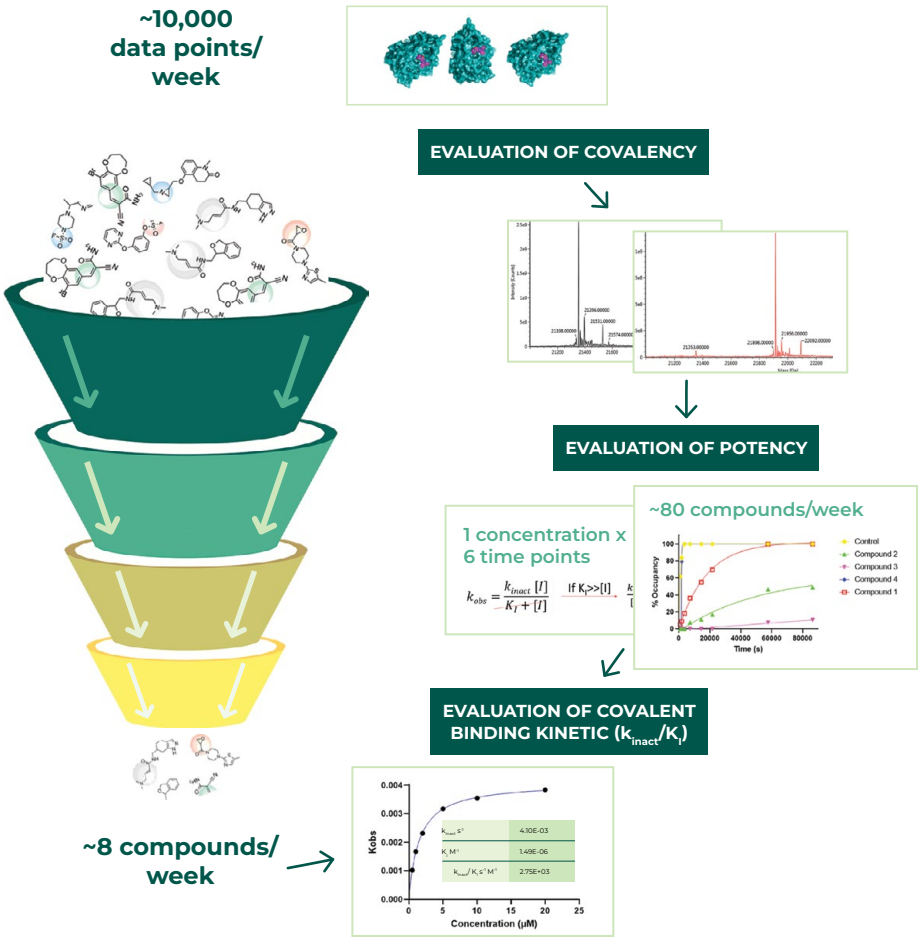
Unveiling and Optimizing Covalent Hits

DALRIADA'S COVALENT LIBRARY

Modular in design, allowing flexibility to target specific nucleophilic residues

Continually updated with new reactive warheads to ensure cutting edge design

Library module sizes are: 3.3K, 8.4 K and 12.5 K



- Ultra high-throughput covalent library screening
- Wide-range screening condition optimization
- Extensive QC and IMA feasibility tests
- GSH Stability Tests
- Secondary platform to screen challenging proteins
- Dalriada's data analysis tool

INTACT MASS ANALYSIS (IMA)

Agilent RapidFire TOF MS

CELL BIOLOGY

Cell-based validation of functionality

- Broad experience focused on the fields of Oncology, GPCRs, Inflammation and Immunology
- Development of robust and relevant custom cell-based and functional assays, such as MOA studies, Cell signaling cascade exploration, Phenotypic screening
- Internal bank of cancer/ normal cell lines, primary cell models, stable/transient cell line generation, 2D/3D cell culture, CL2 labs

Cell Viability/IncuCyte Live Cell Imaging

Cytotoxicity, Proliferation, Apoptosis (Annexin V/PI, cleaved caspases), Immune mediating killing of tumor cells, 3D Spheroid Killing Assay

Targeted Protein Degradation

HiBiT bioluminescence assay, Protein detection by Western blot &. quantitative Western (Jess), Protein Stability, Immunoprecipitation/ Ubiquitinylation assays

Intracellular Target Engagement

NanoBRET, HiBiT CETSA (BiTSA), Jess, Flow Cytometry

Cutting-edge Platforms for Cell-Based Assays

IncuCyte, Flow Cytometry, ELISA, Luminex MagPix, Tecan Spark, Tecan D300e, Formulatrix FAST

Cell-based Functional Assays

Necroptosis rescue assay, Inflammasome, Reporter, IP-One, HTRF, Calcium flux assay

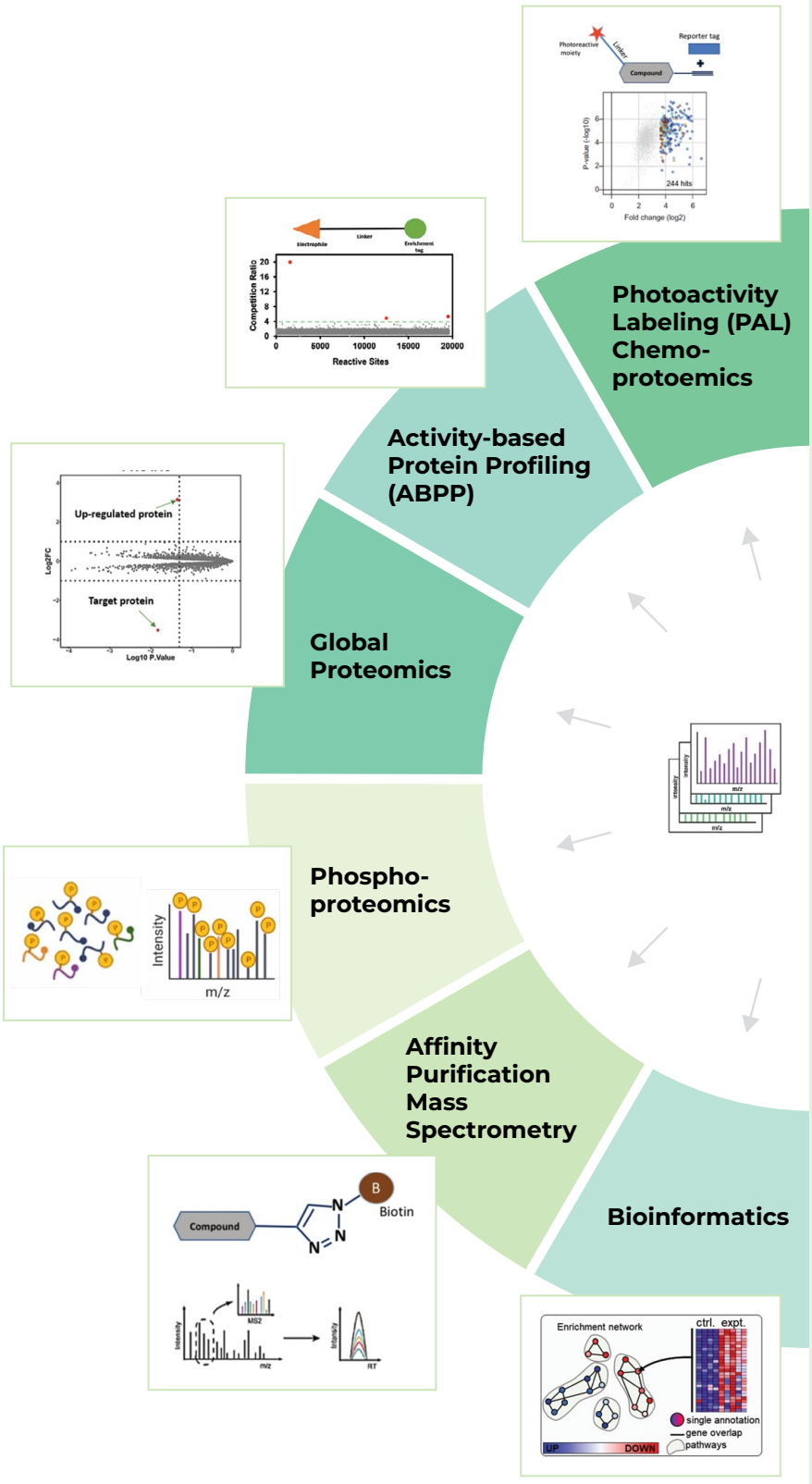
Oxidative Stress Assays (Cellular/ Mitochondrial ROS, MitoSOX), Neurotransmitter uptake inhibition assay (SERT/DAT/NET)

Target Modulation & Validation

RT-PCR, RNAseq, siRNA, shRNA, CRISPR, Overexpression, Immunofluorescent and confocal microscopy

PROTEOMICS

Empowering drug discovery with precision-driven, high-throughput proteomics



Dalriada's proteomics platform combines advanced mass spectrometry with automated bioinformatics for actionable drug discovery insights.

Chemo-Proteomics

Cysteine and N/O ABPP, photoaffinity labeling with end-to-end automation.

Global Proteomics & PTM Profiling

DIA and TMT-based workflows with tunable depth (6,000–9,000 proteins).

Target Deconvolution

Direct targets (Pulldown, PAL, ABPP) and indirect targets (GP, PTM profiling).

Degrader Proteomics

High-throughput screening, whole-proteome turnover profiling and tertiary complex characterization.

Bioinformatics & Data Visualization

Automated data processing, functional enrichment analysis, quality control, and the proprietary iCLASS suite—delivering interactive, actionable insights in just a few clicks.

> Dalriada's expertise and devotion has contributed significantly to the success of our STAT degrader platform and our being able to secure additional financing to further advance the project.

Janpix

> Dalriada's team rapidly designed and delivered bespoke novel primary cell assay system, with multiple inflammatory readouts for effective screening of several candidates and significantly advance our target validation efforts towards our development of next generation therapies in skin diseases.

In4Derm

> Dalriada helped us to expand our footprint in medical cannabis field using pharmaceutical approach. Dalriada far exceeded our expectations by rapidly establishing a drug discovery platform, delivering a substantive IP portfolio and robust pipeline of distinct classes of novel proprietary cannabinoid analogs- which are progressing into pre-clinical development.

CANOPY GROWTH CORPORATION

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