Miniaturized Whole Cell-based GPCR cAMP Assay Using a Novel Detection System

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Overview

- GPCR HTS assays
- ACT:One cAMP biosensor
- Exelixis screening platform
- Case study: CB1 HTS campaign
- Summary

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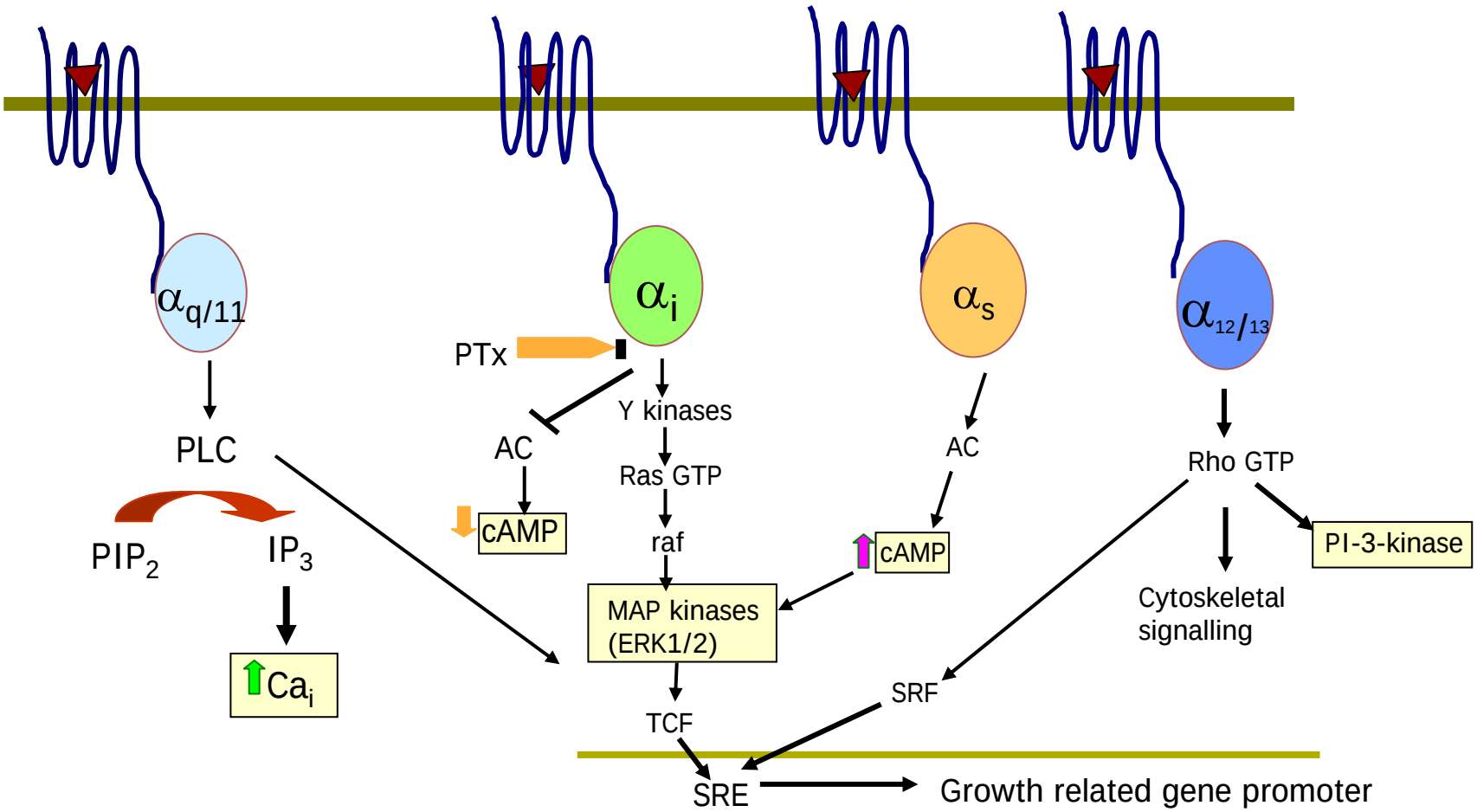
GPCR Signaling Pathways



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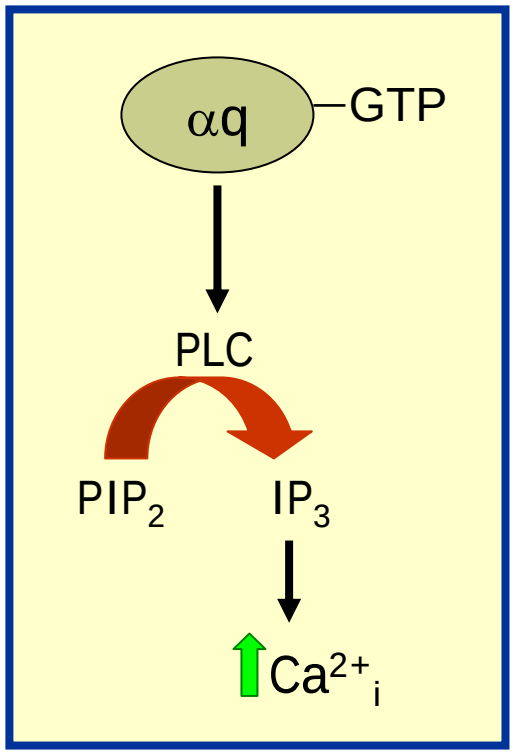
Gq and Calcium Signaling



- Fluorescence Imaging Plate Reader
- Load cells with calcium sensitive dye
- Changes in fluorescence correlate directly with changes in intracellular calcium
- 384-well format

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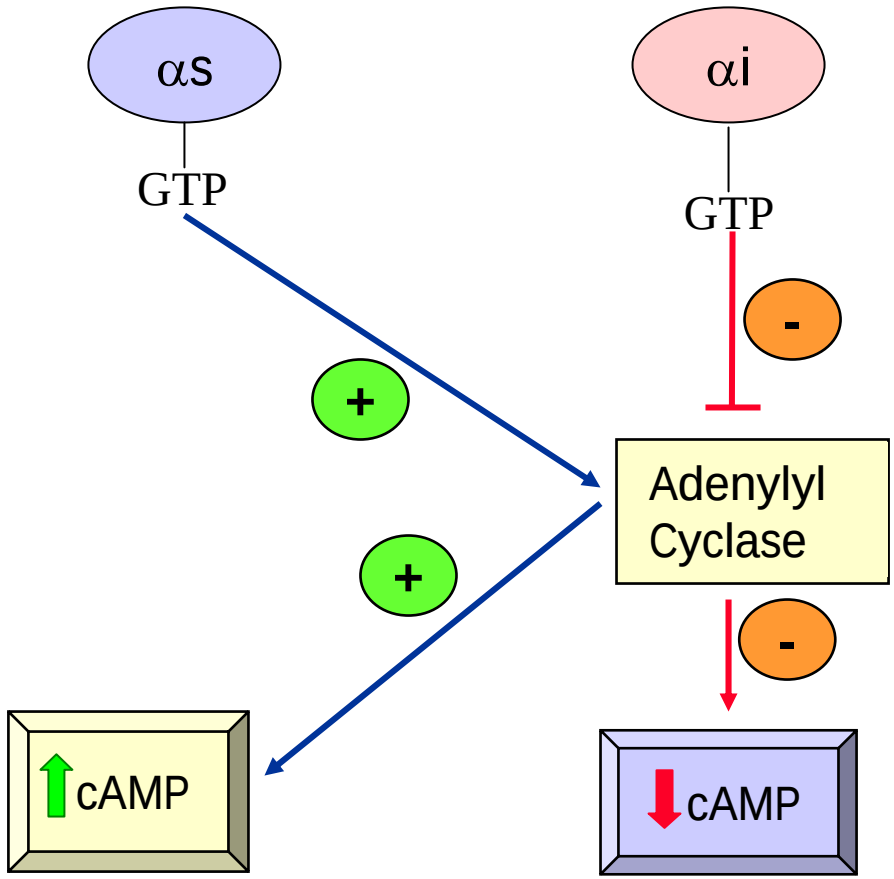
Gi and Gs-coupled receptors



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HTS strategies for non-Gq coupled receptors

- Chimeric Gα proteins: Gqi5, Gqs, Gqx
  - Couple to Gα as normal, but signal like Gq (via calcium)
  - FLIPR adaptable
- Promiscuous Gα proteins
  - Gα15/16: Overexpress to drive signaling through calcium pathway
- cAMP assays for Gi and Gs coupled
  - Radioactive methods
  - FP
  - AlphaScreen
  - Enzyme complementation assays

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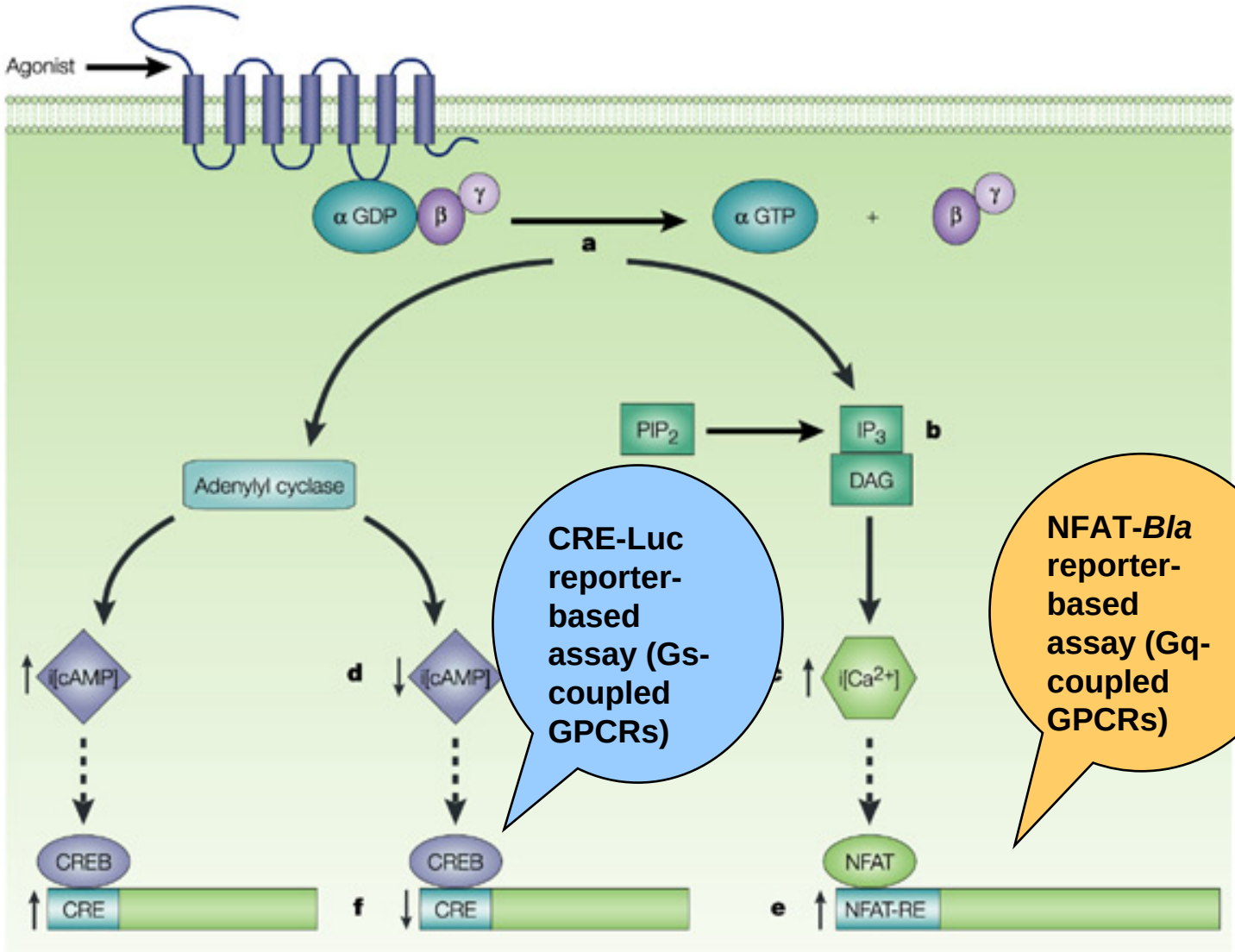
Reporter-based assays for GPCRs



Nature Reviews | Drug Discovery

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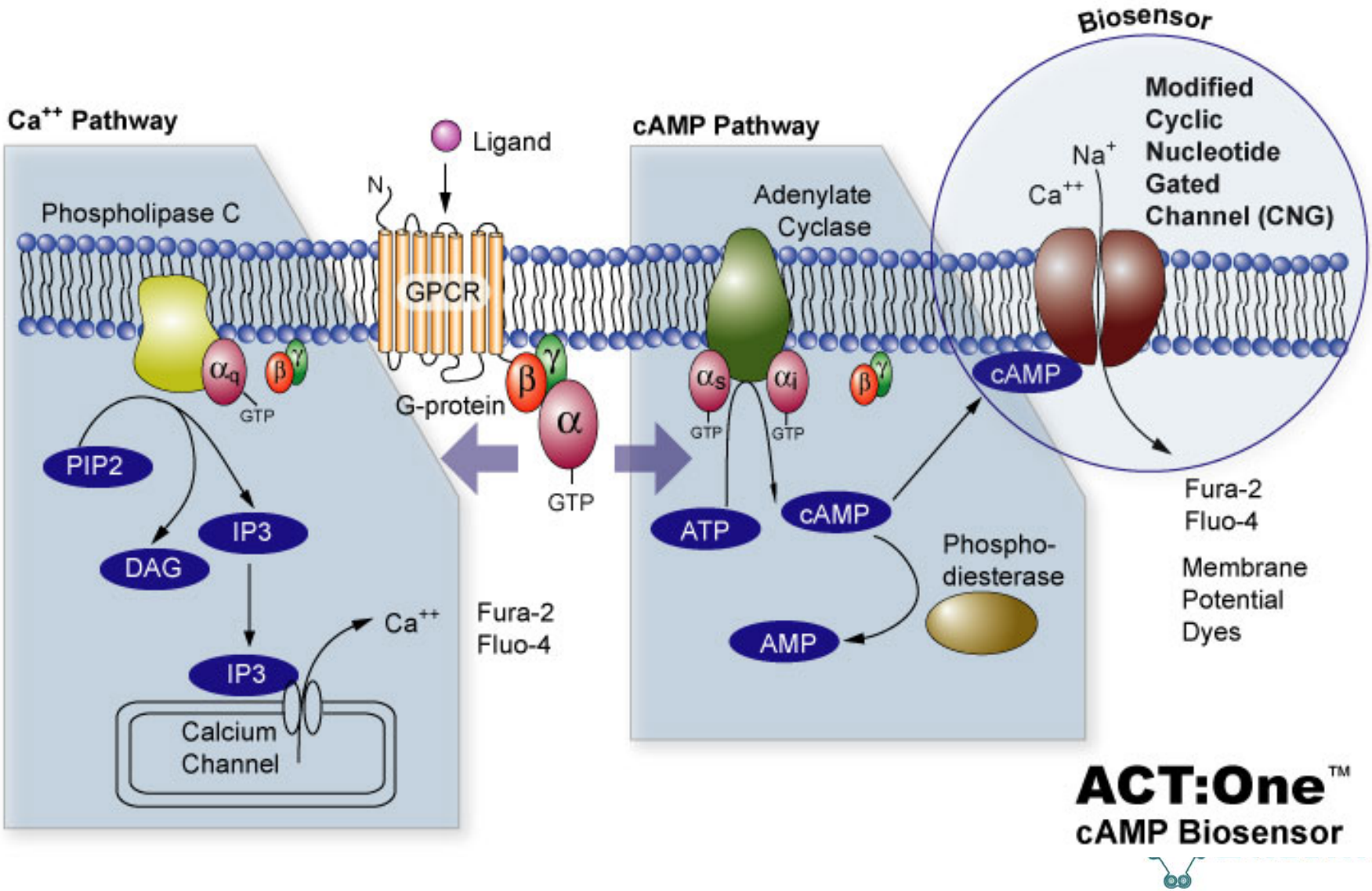
ACT:One Biosensor



Biosensor based cAMP assay

- No modification of GPCRs required
- Endogenous and overexpressed receptors
- Gi and Gs coupled GPCRS
- Agonists and antagonists
- No radioactivity; fluorescence read-out (MP dye)
- End-point or kinetic measurements
  - Stable signal
  - Automation friendly
  - No special equipment/reader

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Example: CB1 HTS campaign using CNG biosensor technology

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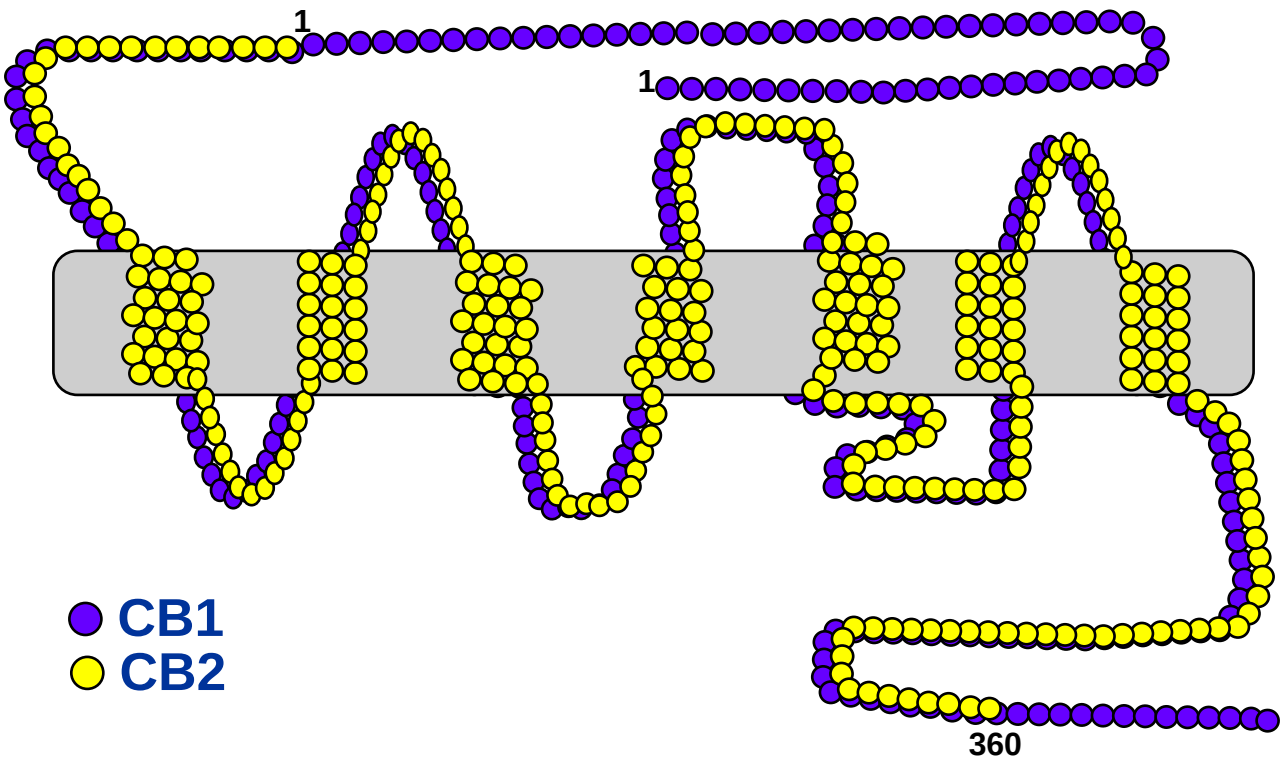
Cannabinoid Receptor 1 Project

- Goal: Discovery of a novel, orally-bioavailable CB1 antagonist for the treatment of obesity
- Clinically-validated target – Rimonabant™
  - Phase 3 (Sanofi-Aventis)



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The EC System Is a General Stress-Recovery System and Is Overall "Silent"; It Becomes Transiently Activated To:

Relax

reduction of pain and anxiety; modulation of body temperature, hormone production, smooth muscle tone, and blood pressure

Rest

inhibition of motor behavior and sedation

Forget

extinction of aversive memories

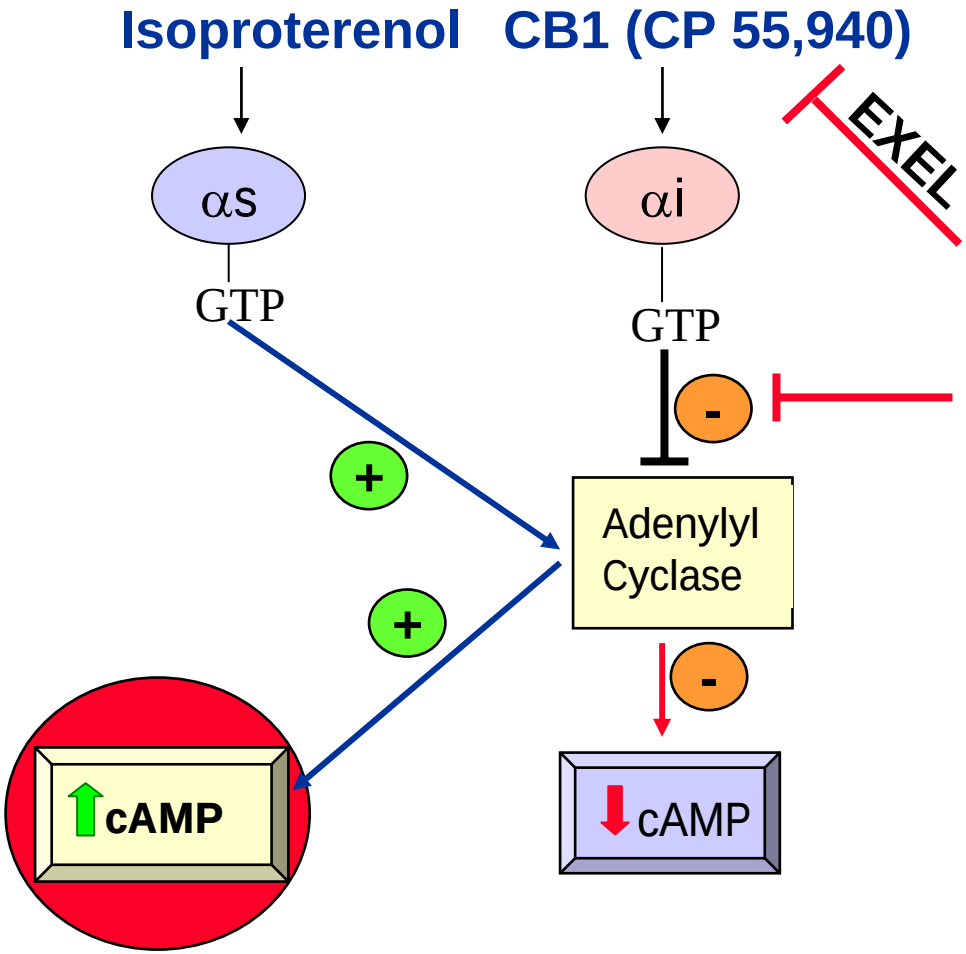
Protect

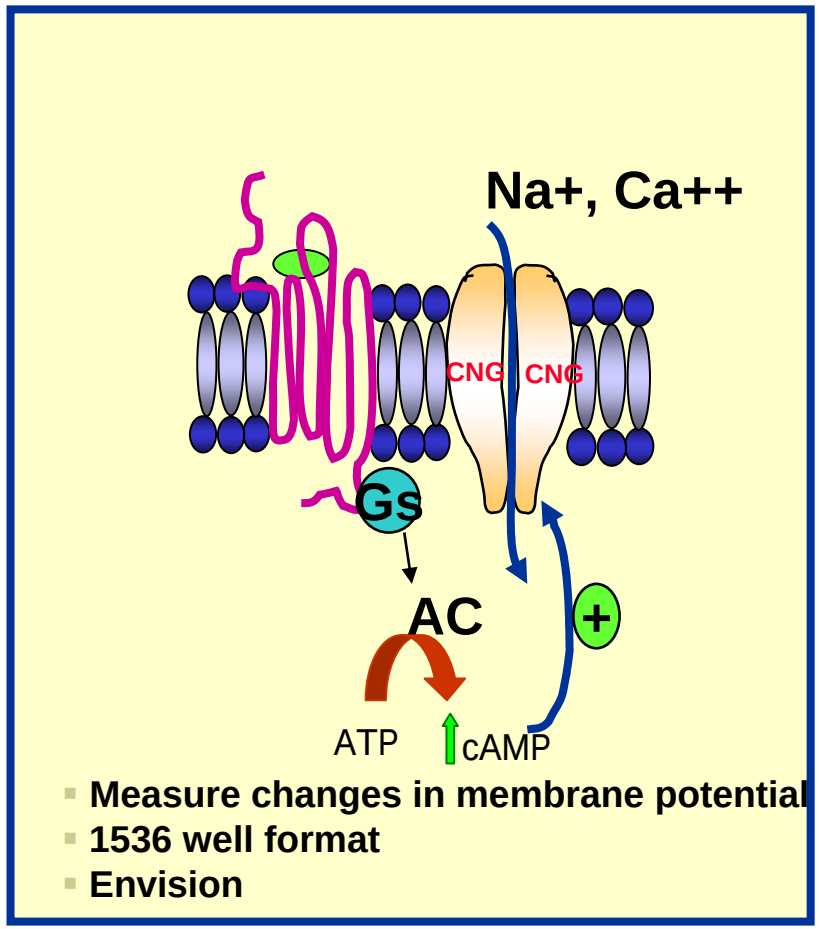
at both the cellular and emotional levels

Eat

appetite-inducing and reward-reinforcing effects

CB1 – Gi-coupled receptor





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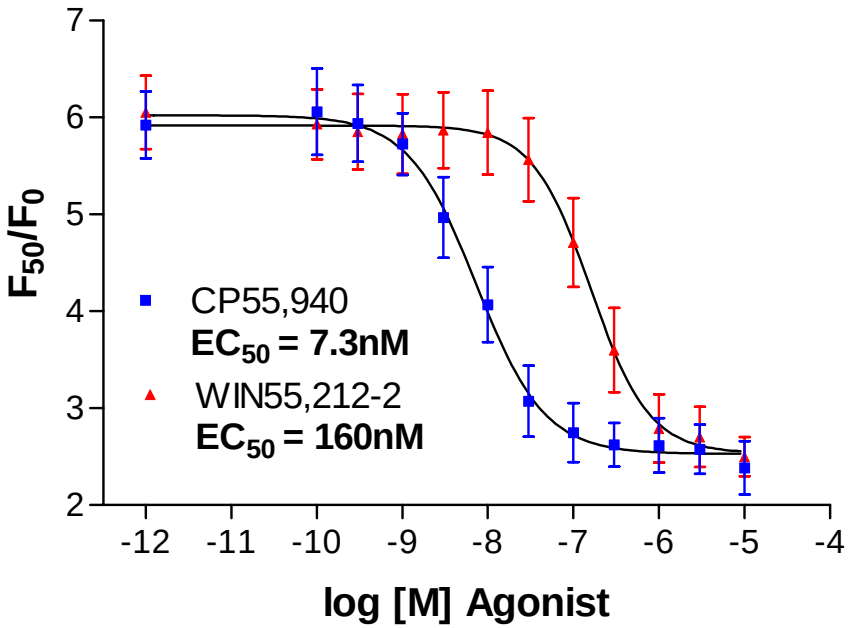
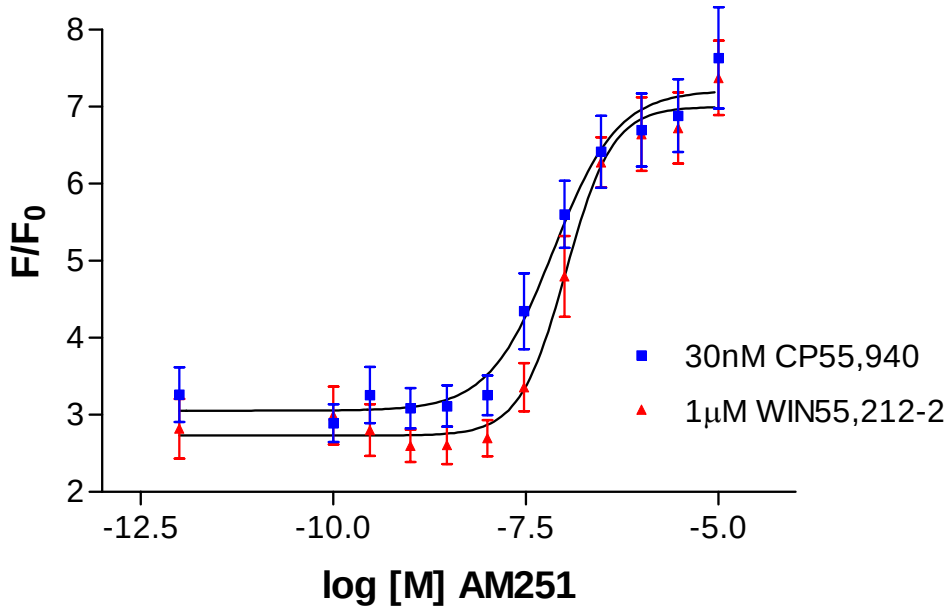
CB1 Pharmacology: Act:One cAMP Assay

1536-well format



Agonist stimulation



Inhibition by AM251

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High Throughput Screening Platform

- HTS is not capacity limiting
  - Routine screening of 4+ MM cmpds
- Dynamic technology
  - 384/1536 microtiter plate formats
- Continued increase in library size
  - Complete HTS in 1-2 weeks

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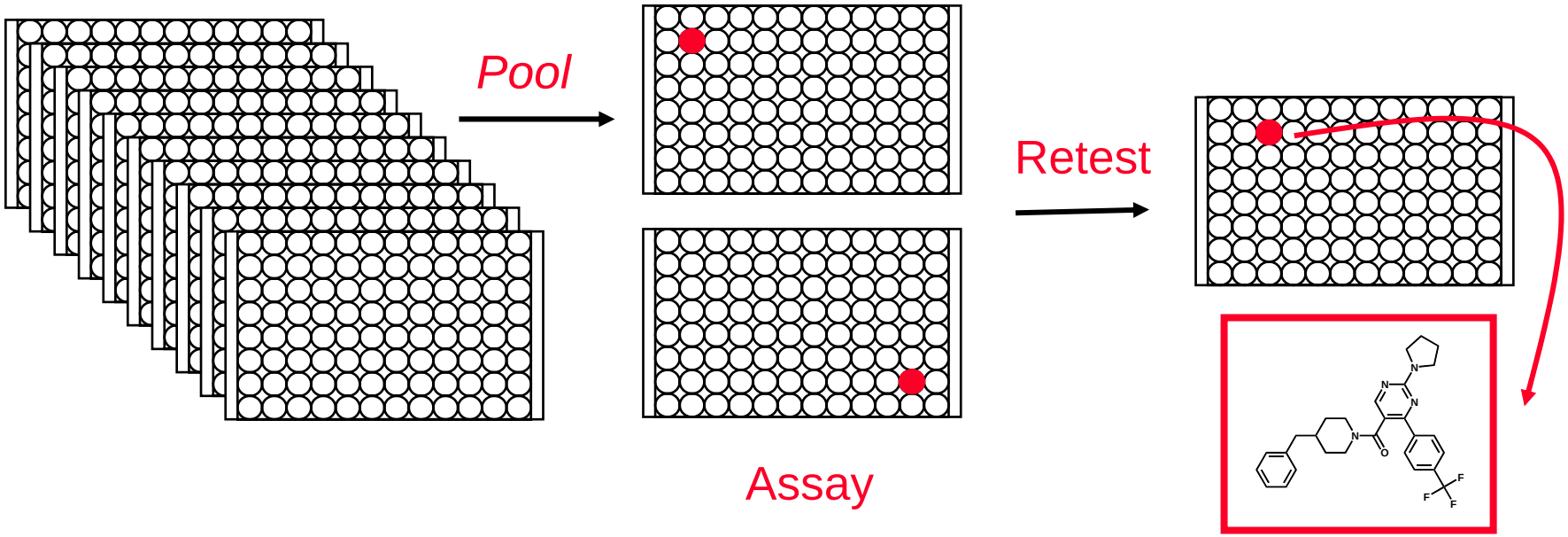
Orthogonal Compound Pooling



- 10 Compounds/well in two sets of mixtures
- Deconvolute replicates and confirm as single compounds
- Low false positive and false negative hit rate

Five-fold increase in HTS efficiency

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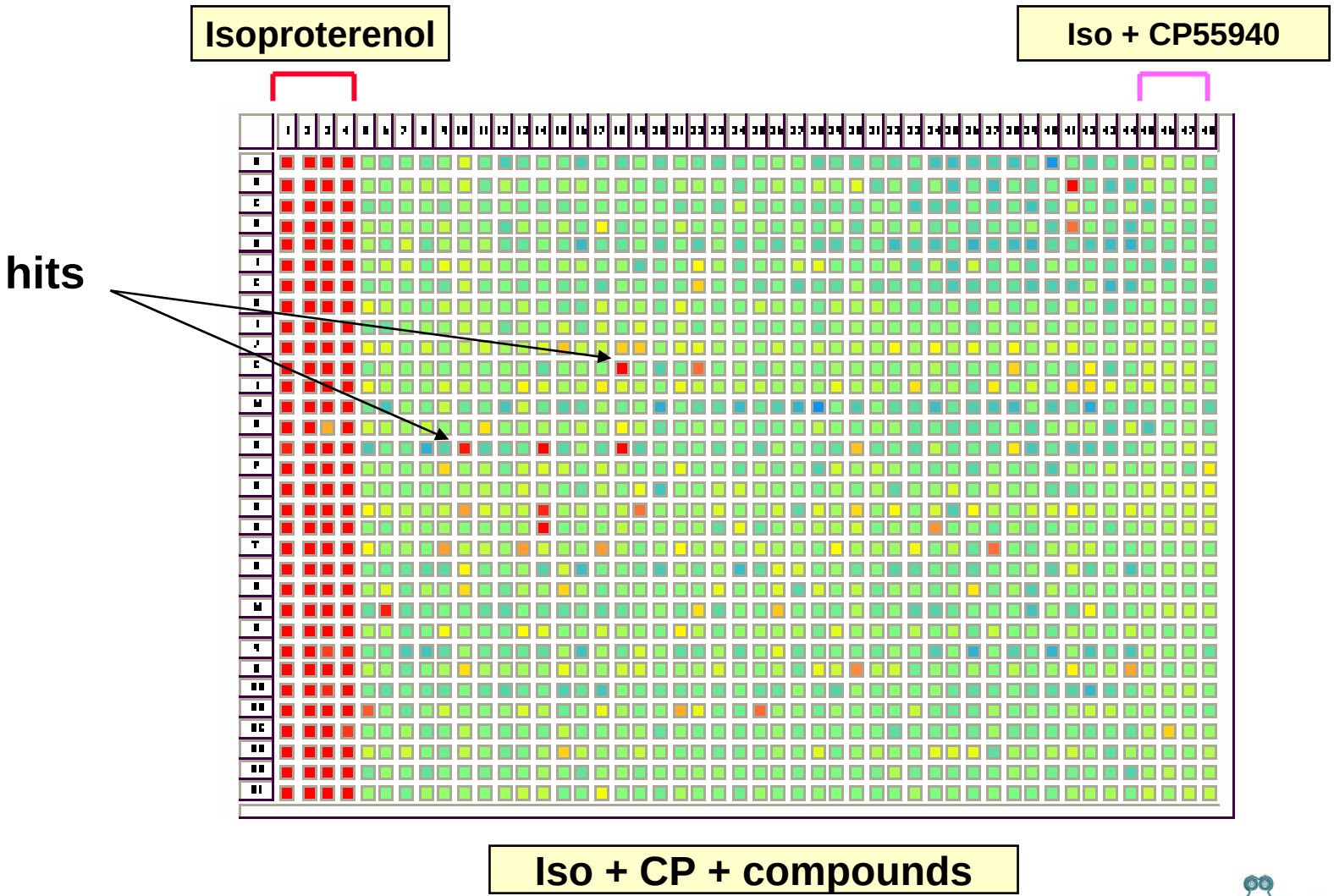
cAMP assay format



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HTS Assay Performance

- 3.6 M Compounds screened at 1.7 μM
- 1536-well format
- 4000 cells/well; 50 minute incubation; end-point fluorescence read
- Novel, robust assay format for G_i-coupled receptors
  - Z' = 0.4 – 0.6
  - Mean S/B = 2.2
  - # hits = >25,000 (75-100%)
- Identified multiple potent antagonists

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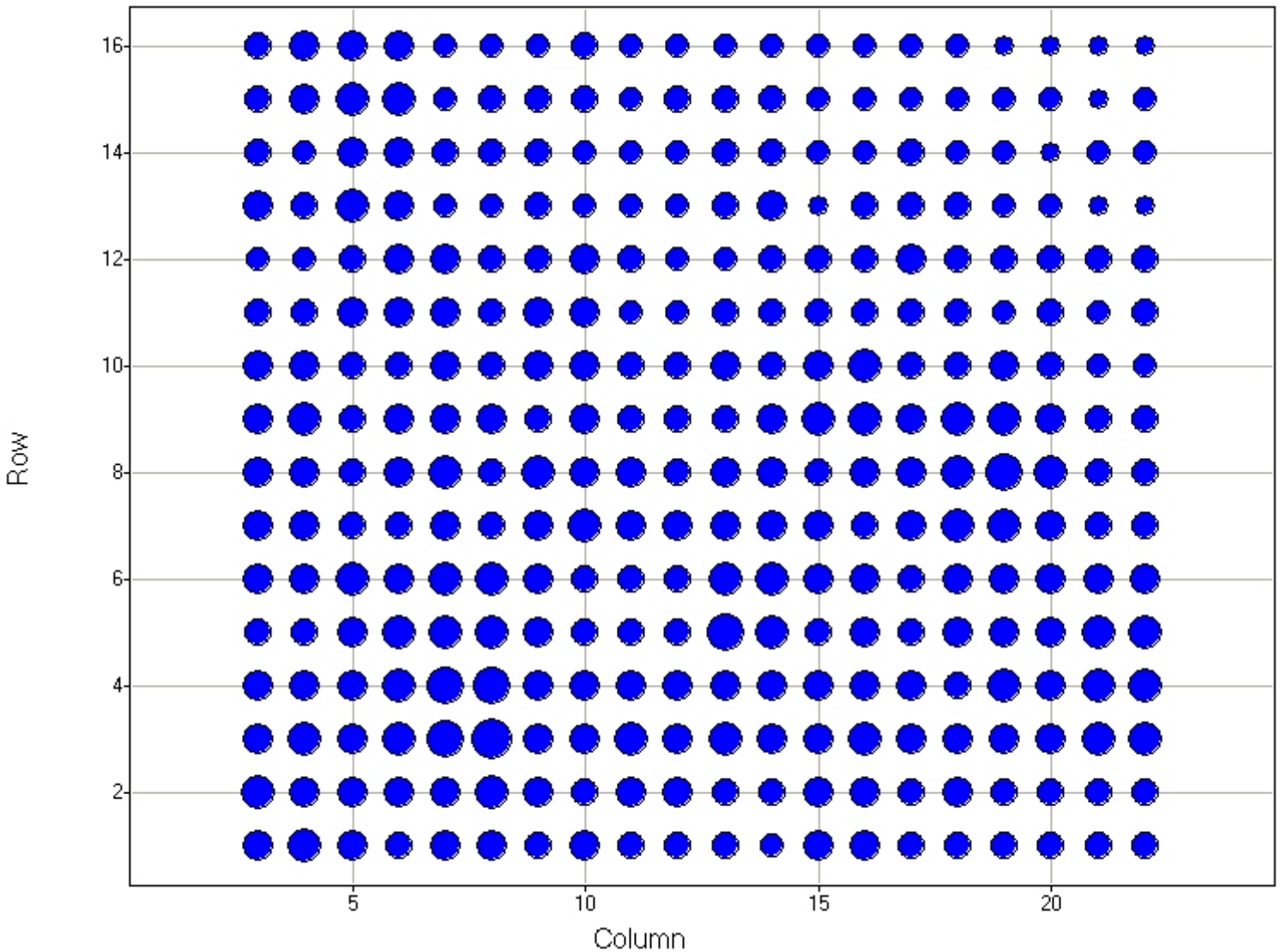
Hit distribution



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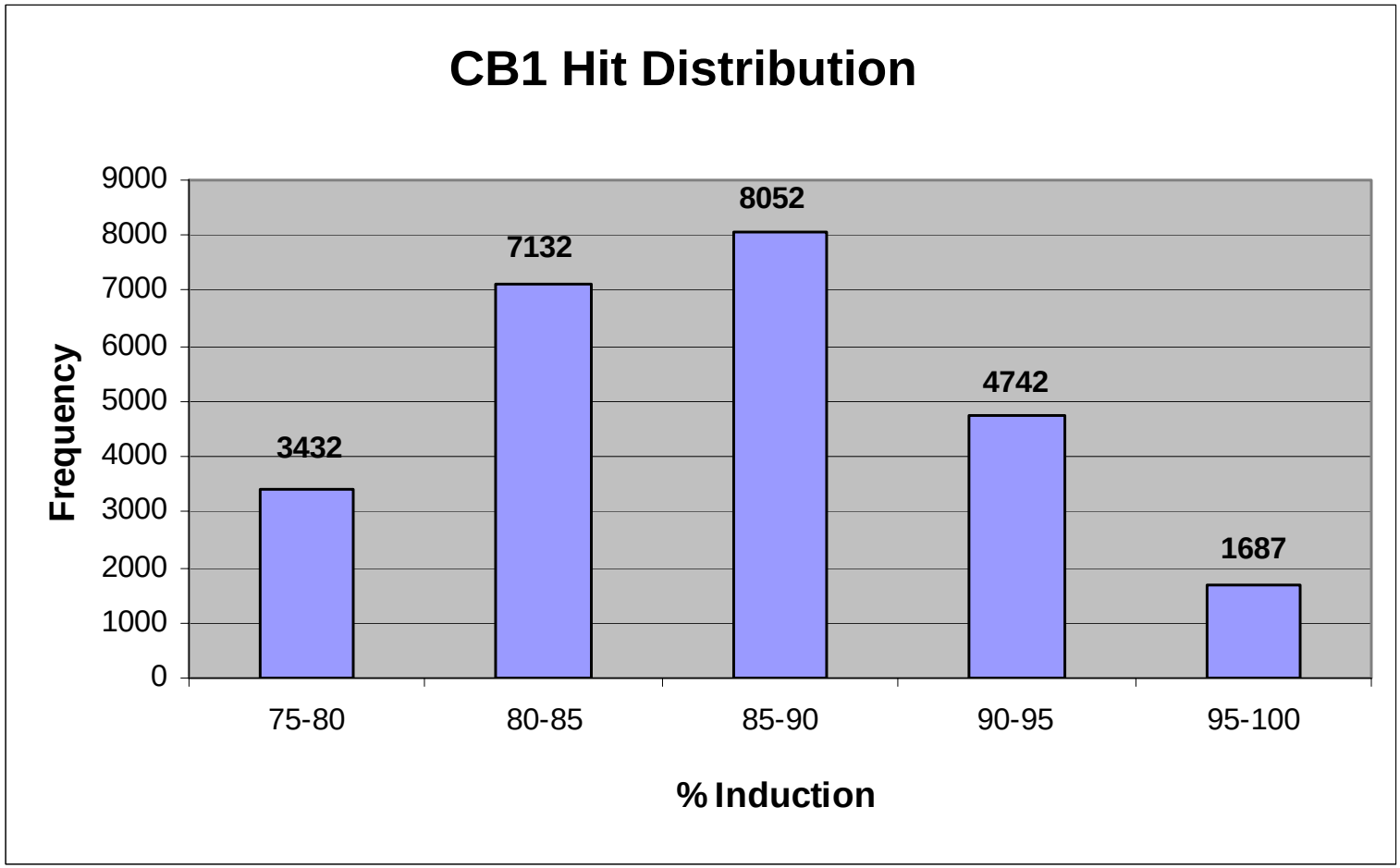
Hit distribution



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Confirmation Summary

- 667 Compounds confirmed upon single point retesting
- 208 Compounds evaluated in dose-response studies
  - 4 < 10 nM, 73 <100 nM, 123 <500 nM IC_{50} (cAMP)
- Eleven scaffolds confirmed via resynthesis
  - IC_{50} values 1-200 nM
  - >60% derived from internal combi-chem synthesis
- PD studies used to prioritize lead series

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Summary of Confirmed Actives

Compound (# examples)	CB1 – cAMP (nM)	CB1 – Ki (nM)	PD (100 mpk) % reversal
Rimonabant	4	3	80 (30 mpk)
EXEL-3627 *(85)*	1.5		79 (300 mpk)
EXEL-9628 *(17)*	3.2	>100	8 (300 mpk)
EXEL-5412 *(20)*	3.8	-	-
EXEL-4933 *(62)*	4	50	36
EXEL-3124 *(45)*	8	-	-
EXEL-2355 *(52)*	13		31 (300 mpk)
EXEL-7594 *(1)*	23	76	3
EXEL-5450 *(13)*	77		-40 (300 mpk)
EXEL-5649 *(8)*	100	-	-
EXEL-4197 *(10)*	107	-	-
EXEL-6921 *(6)*	183	-	-

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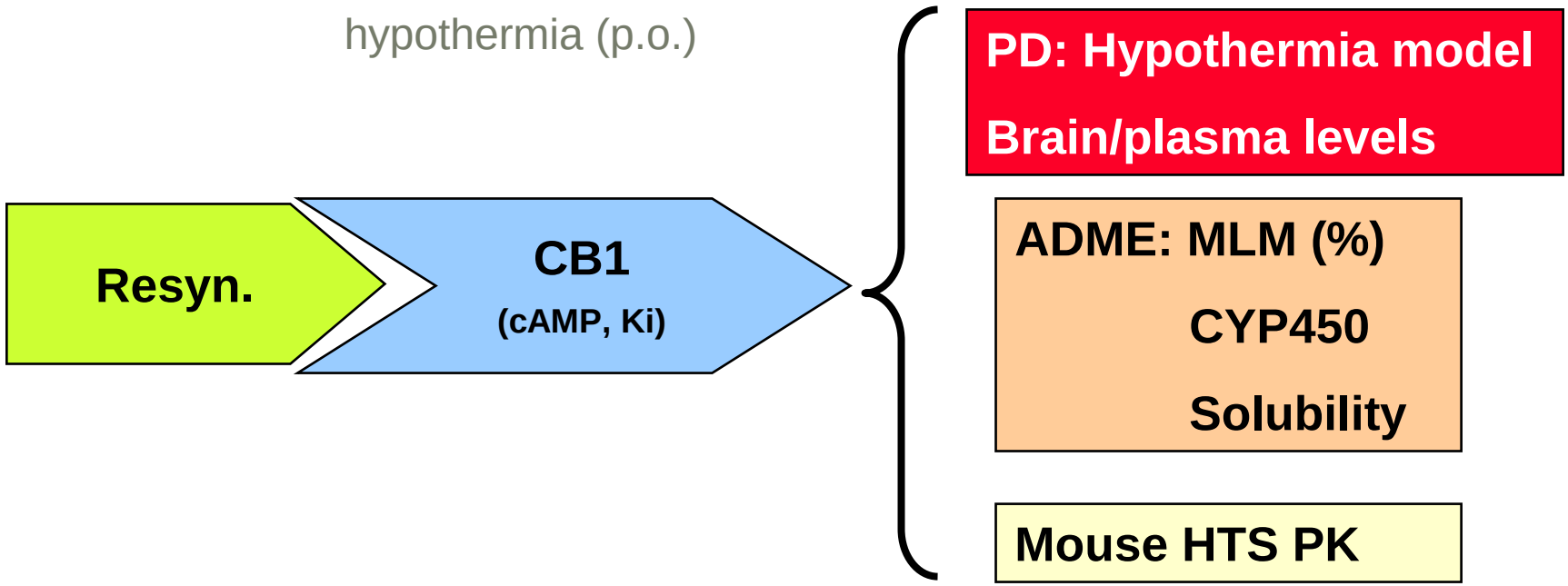
Lead Validation

- **Compound Progression**
  - Active resynthesized HTS compounds
    - cAMP, Displacement binding (<10 nM)
  - Pharmacodynamic assay
    - Reversal of CB1-agonist induced hypothermia (p.o.)



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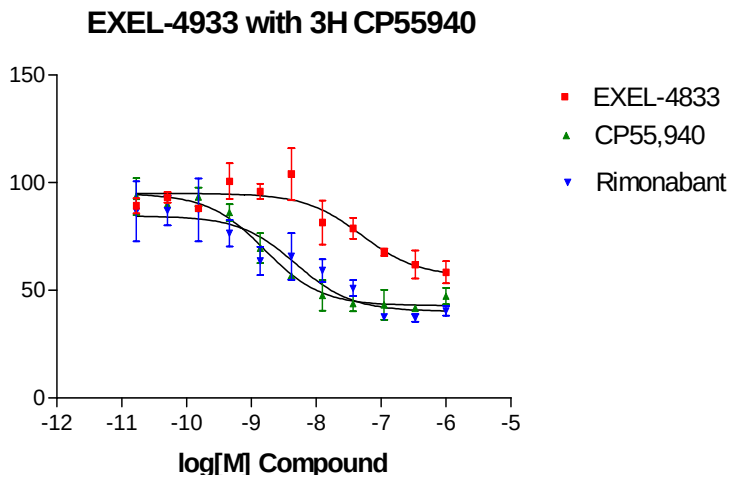
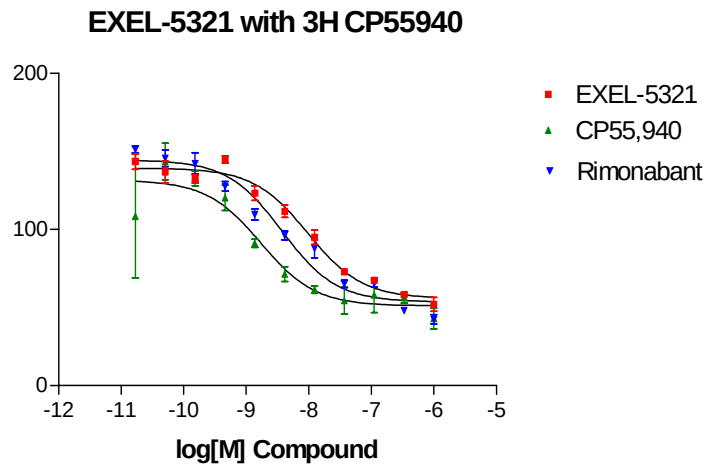
In vitro displacement binding

SAR-dependent binding observed



EC_{50}: 8 nM

Ki: 50 nM



EC_{50}: 5 nM

Ki: 10 nM

Correlation with in vivo efficacy

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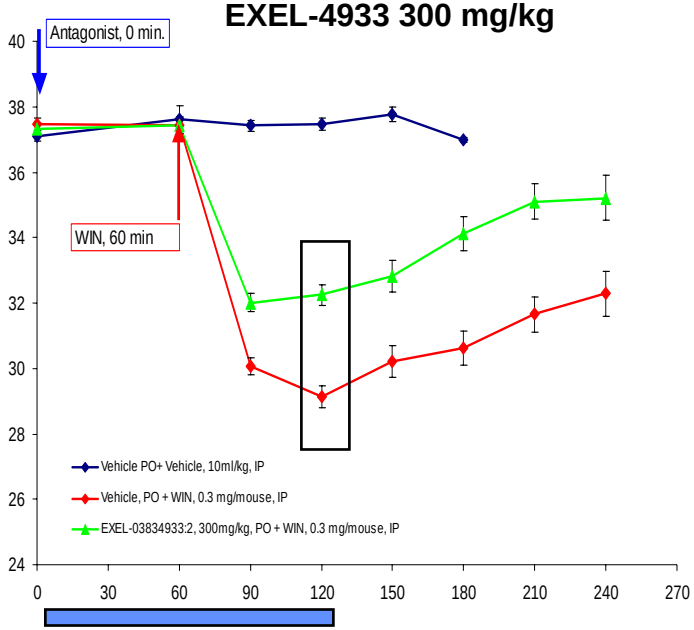
EXEL-4933 mediated prevention of hypothermia

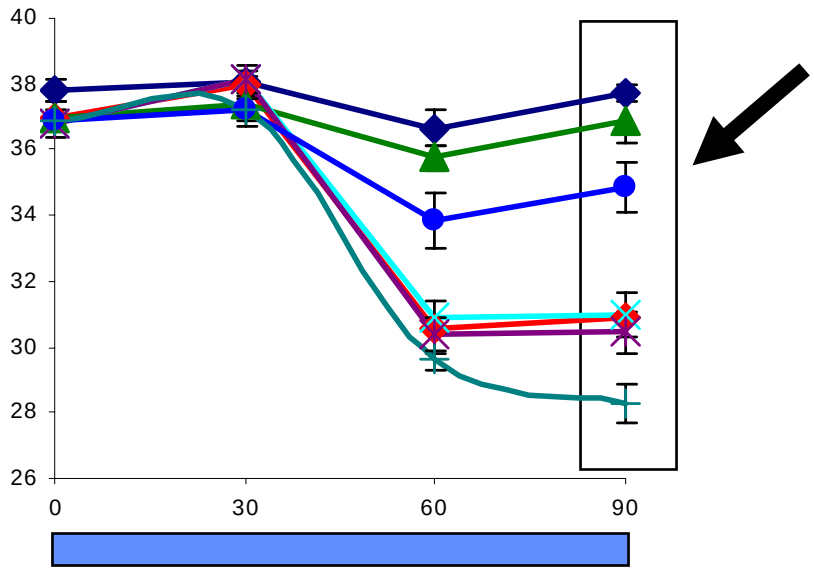
- Validated model mediated by the cannabinoid system

EXEL-4933 300 mg/kg



37% inhibition of hypothermia

EXEL-5321 100 mg/kg



68% inhibition of hypothermia

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Summary

- CNG channel technology offers a novel detection method for cAMP measurements
- Technology has wide applicability to Gi and Gs coupled GPCRs
- Significant cost-savings with miniaturization
- Stable, robust signal allows for detection of potent hits

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Acknowledgments

- GPCR
  - Scott Ogus
  - John Lesnick
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