MRC Technology
Centre for Therapeutics Discovery

Identification and characterization of allosteric modulators of GPCRs: The utility of HTRF and incorporation into generalised screening strategies

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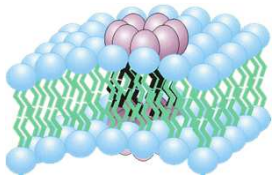
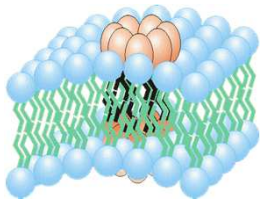
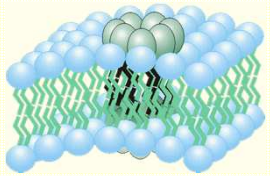
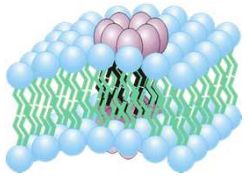
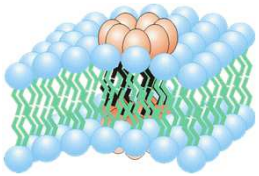
ELRIG – Cisbio Workshop

Sep 2012

Presentation Outline

- Melanocortin receptors
- Allosteric modulation of 7TM
- HTS and compound profiling considerations
  - *Major challenges*
  - *Suggested PAM screening strategies*
- Comparative Pharmacology
- Summary

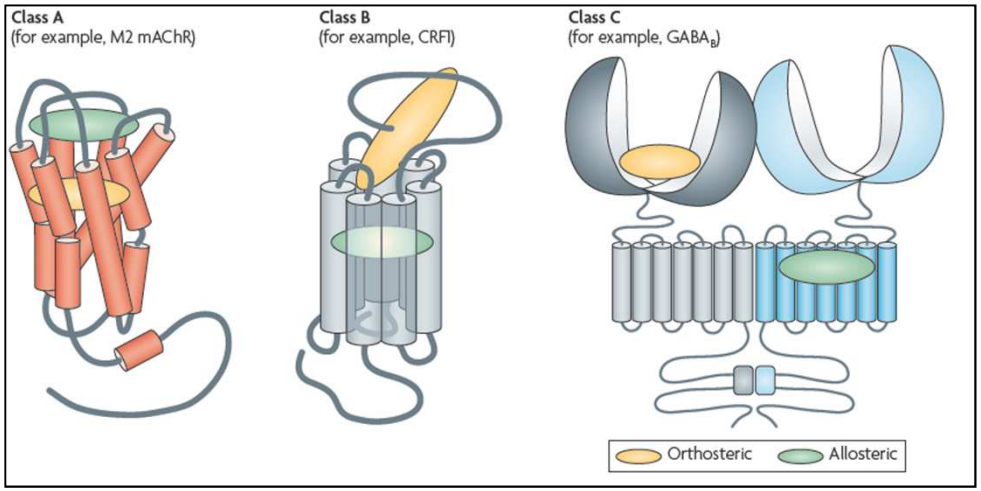
Melanocortin receptors

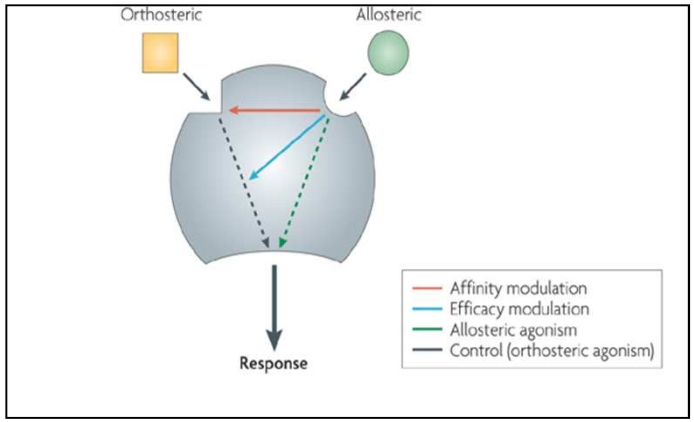
MC_1	MC_2	MC_3	MC_4	MC_5
γ -MSH ACTH α -MSH	ACTH	γ -MSH ACTH α -MSH	γ-MSH α-MSH = ACTH	γ -MSH ACTH α -MSH
				
Melanocyte Endothelial Cells Fibroblast Monocytes	Adrenal Cortex Adipocytes	Macrophages Brain Gut Placenta	Brain	Brain Peripheral Tissues
Pigmentation Inflammation	Steroidogenesis	Cardiovascular Function, Inflammation	Feeding Control (obesity)	Control of the Sebaceous Gland

MC3 receptor *stimulators* are predicted to drive resolution of inflammation

Targeting novel compound mechanism of action

- Orthosteric vs Allosteric binding/functional modality

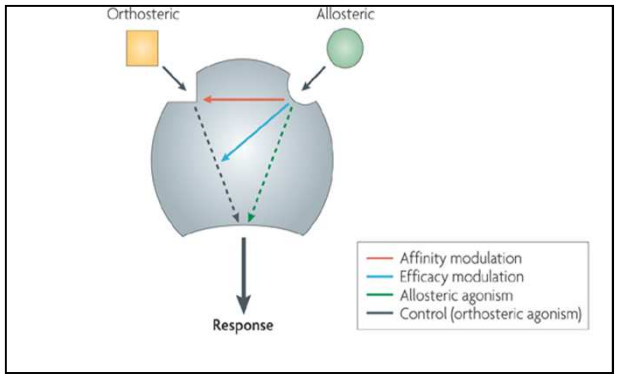


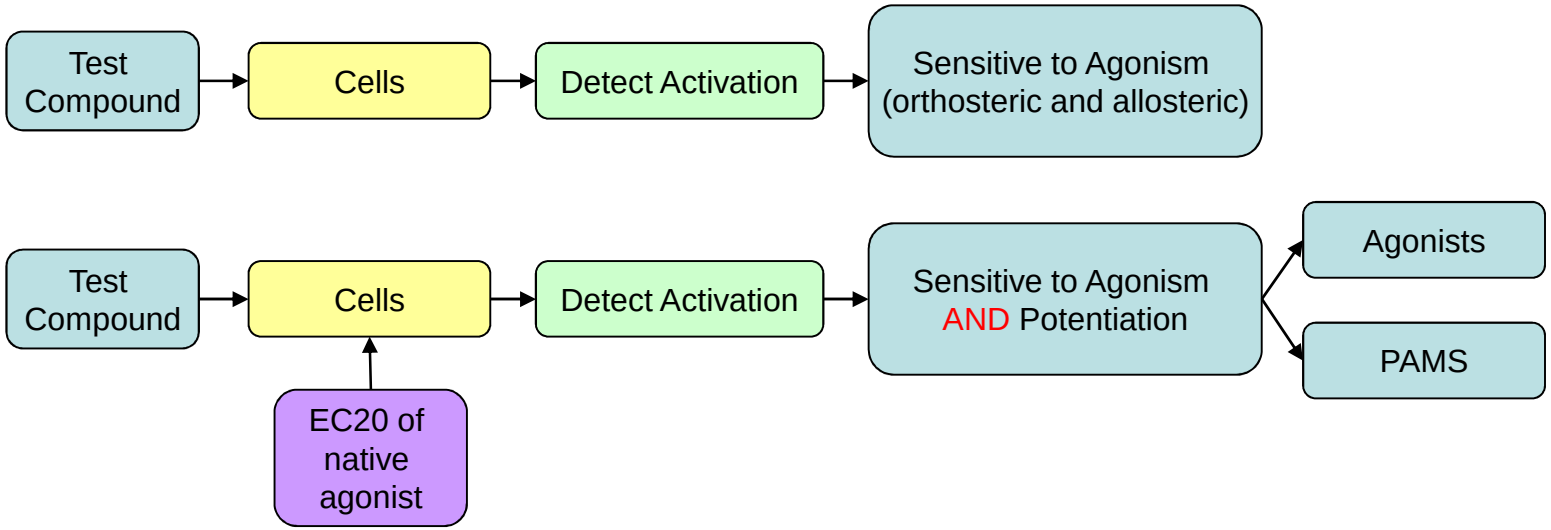


- Advantages of Positive Allosteric Modulation (PAM)
  1. *Improved selectivity*
  2. *Saturability (self-limiting) biological effect*
  3. *Temporal and spatial resolution*

Agonist vs PAM Assay configurations

- 7TM HTS can be configured to detected both agonism and Positive Allosteric modulation simultaneously
  - *The 'simple' inclusion of a submaximal (EC20) of agonist facilitates this*

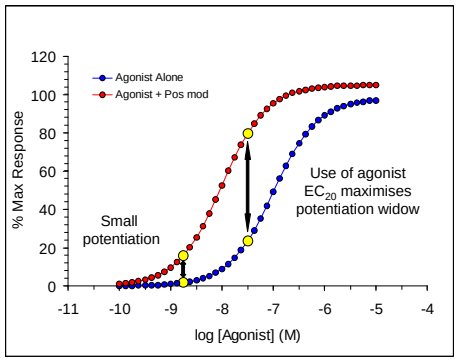




7TM PAM HTS/CP – Major Challenges (1)

Prediction and control of an EC20 stimulus

- *Endogenous melanocortin agonists are 'sticky' peptides (loss and/or carry over)*
- *Changes in receptor expression/coupling can dramatically affect pEC50*
- *The predictability and stability of the EC20 determines the sensitivity to PAMS*
- *HTRF affords both sensitivity and stability in response*



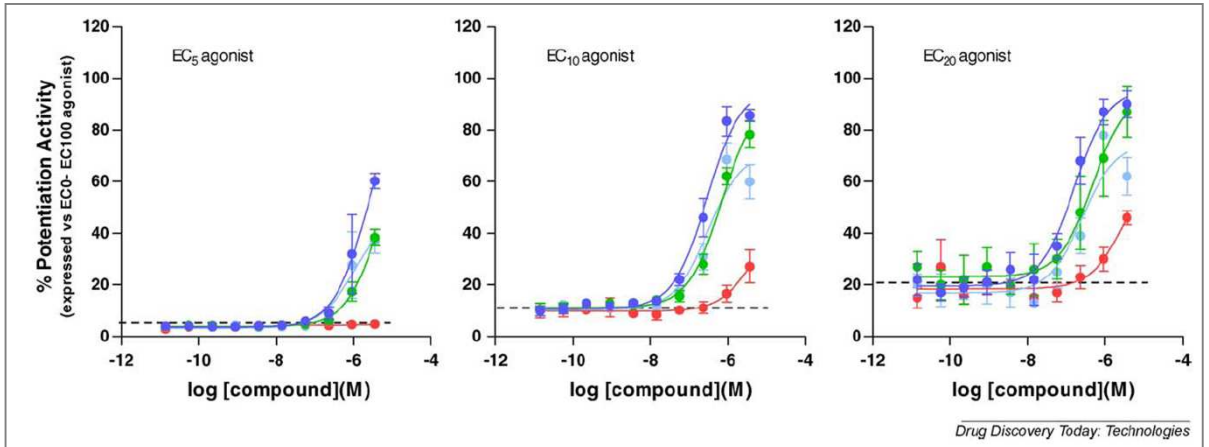
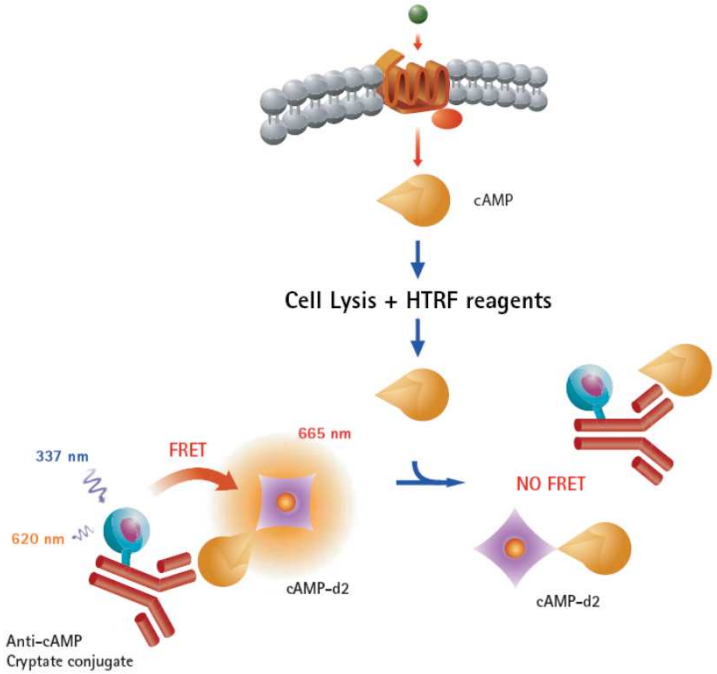
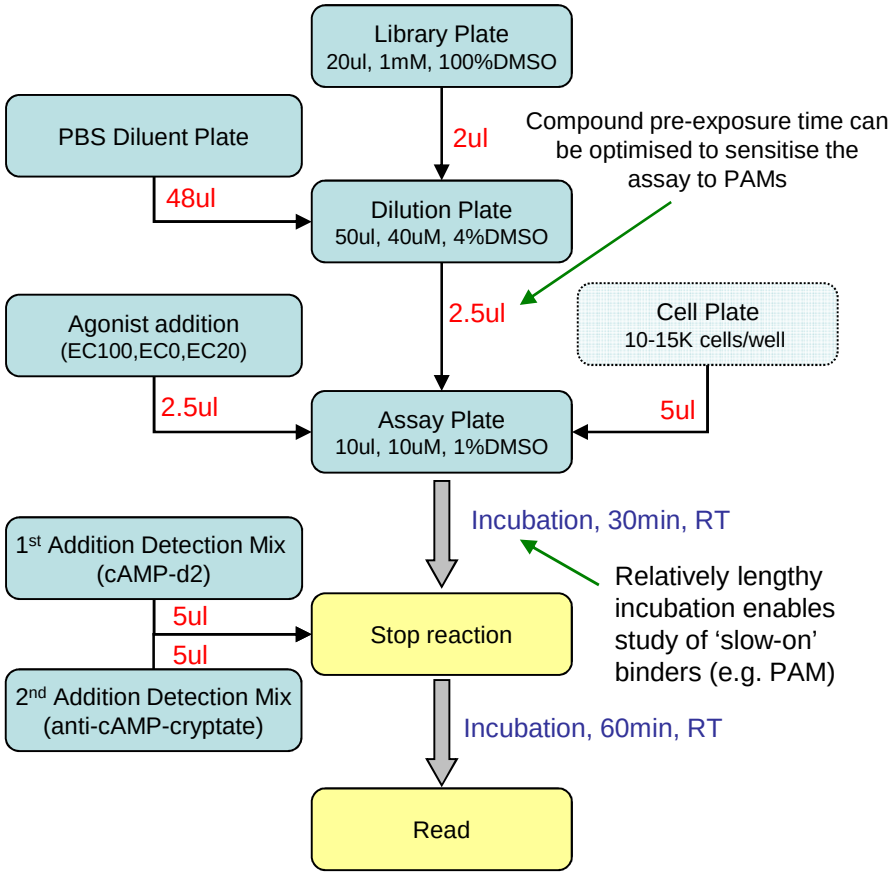


Figure 2. During screening, PAMs are typically tested as a function of a fixed agonist concentration corresponding to EC_{20}. The variability inherent to the measure of pEC_{50} of modulation in production screening can often be under-estimated. The impact associated with using lower than anticipated agonist concentrations over plate runs and/or days on assay sensitivity is significant. The graphs below illustrate the differential modulatory profiles obtained for four compounds derived from the same chemotype series when tested at agonist EC_5, EC_{10} and EC_{20}. Both potency and efficacy values appear to be affected to a different extent for each compound. In particular, it can be noted that one of the compounds is inactive at the lowest condition of agonist.

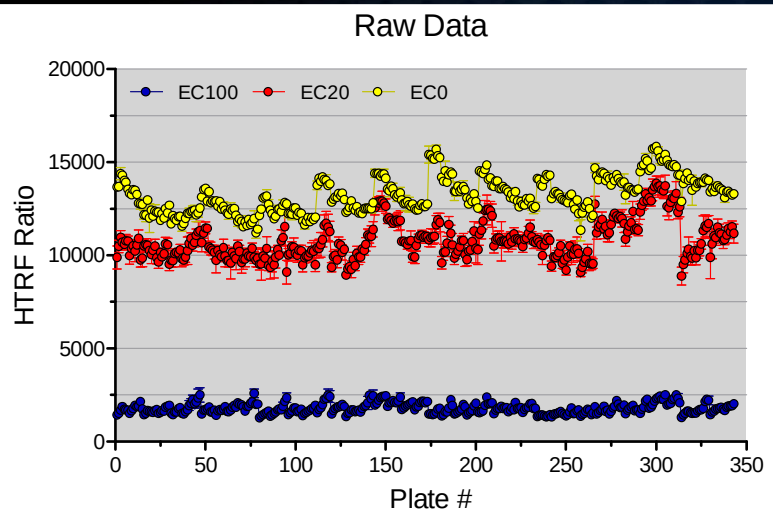
Automated Assay Protocol

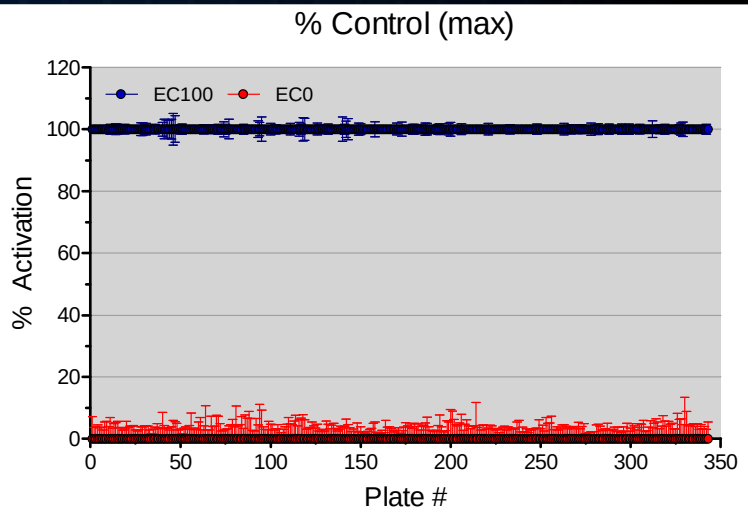
Agonist-induced cAMP accumulation (generic HTRF automation protocol)

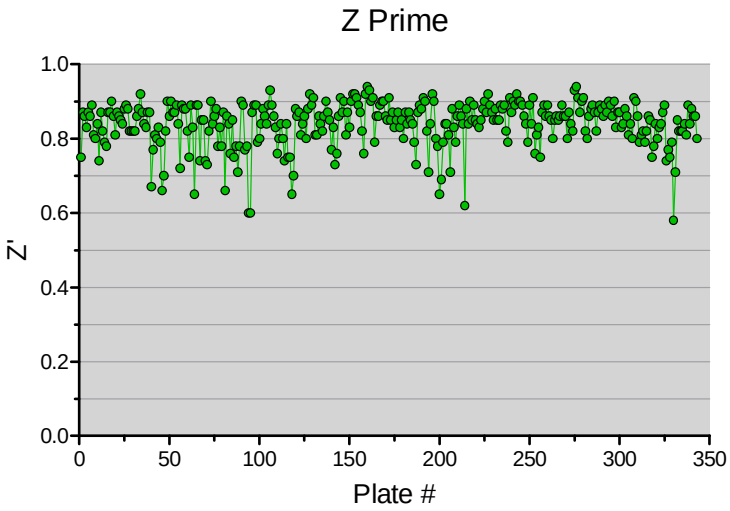


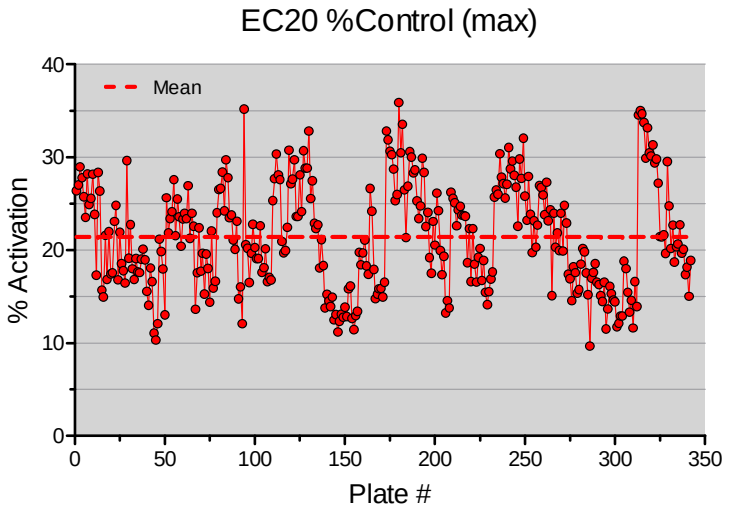


HTS Performance

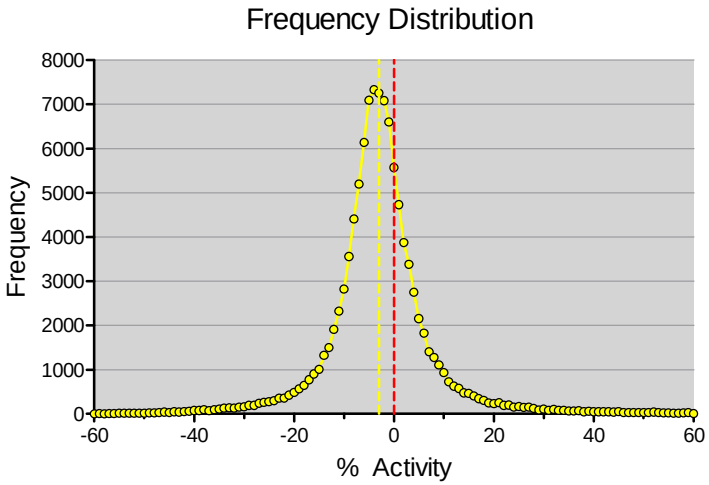


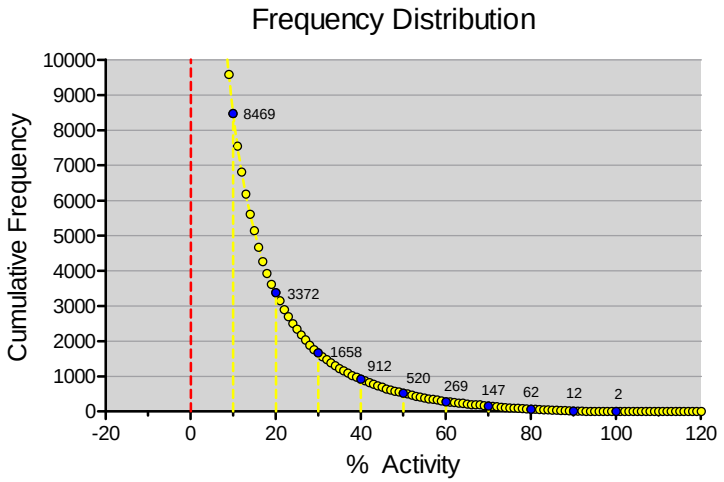


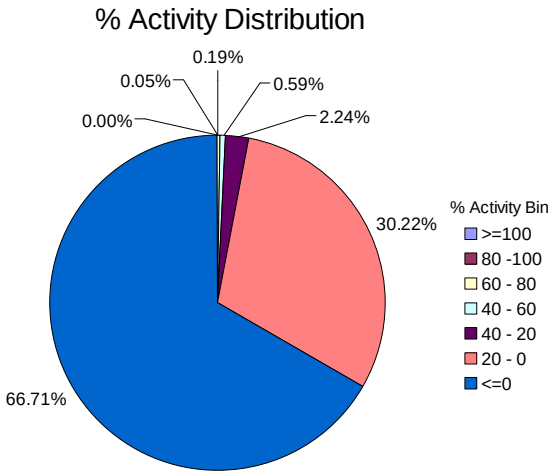




HTS Performance







109760 compounds @ 10mM (1% DMSO)

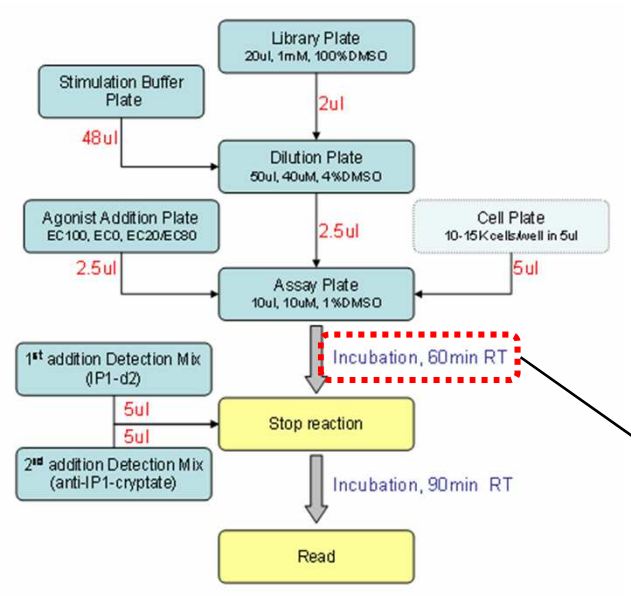
Mean Z' = 0.84 (±0.06)

Low Control %CV = 5.3 (±2.7)

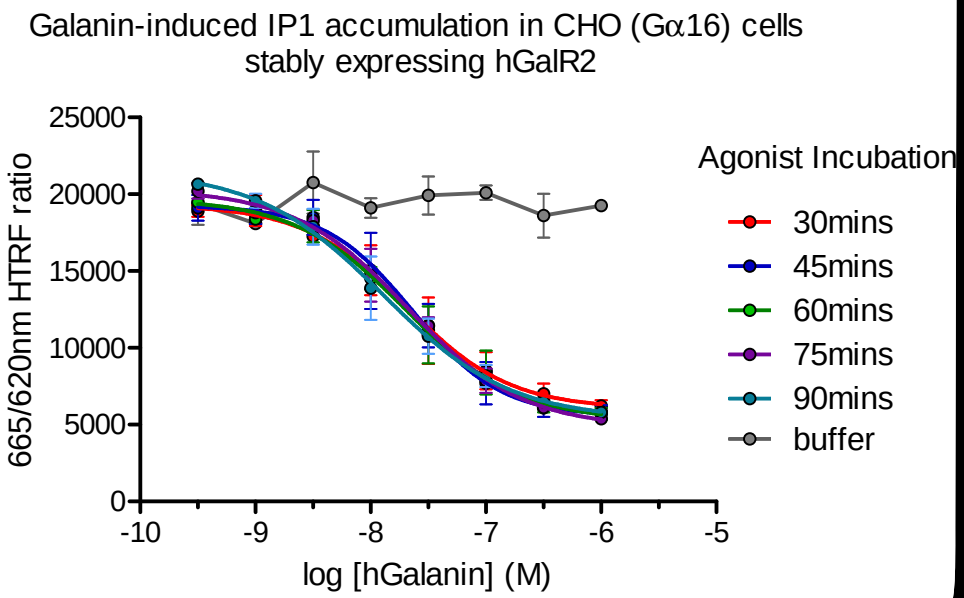
High Control %CV= 3.6 (±1.5)

Cutoff(%)	# Hits	% HR
40	912	0.83
50	520	0.47
60	269	0.25
70	147	0.13
80	62	0.06
90	12	0.01
100	2	0.00

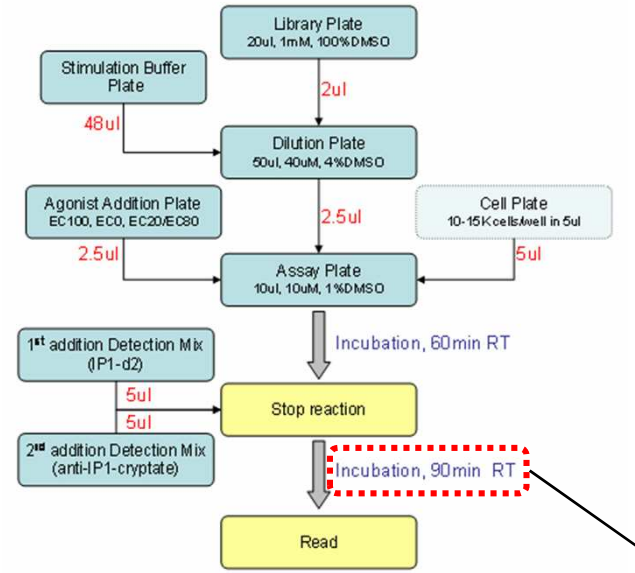
GalR2 IP1 HTS – Agonist incubation time



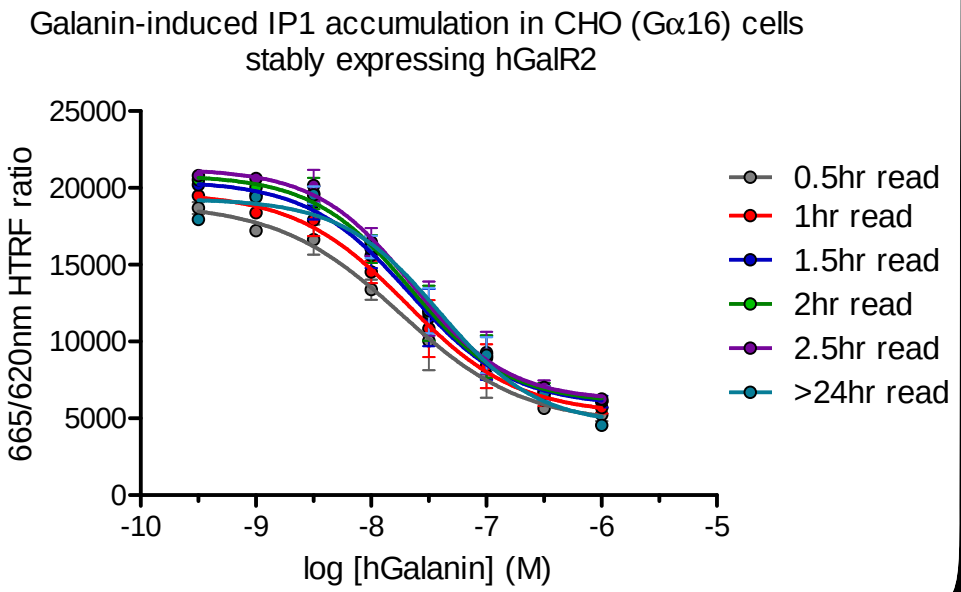
- Agonist pEC_{50} is not dependant on incubation time (within these limits)
- This suggests an EC_{20} should be stable throughout an HTS run/day



GalR2 IP1 HTS – Detection reagent stability



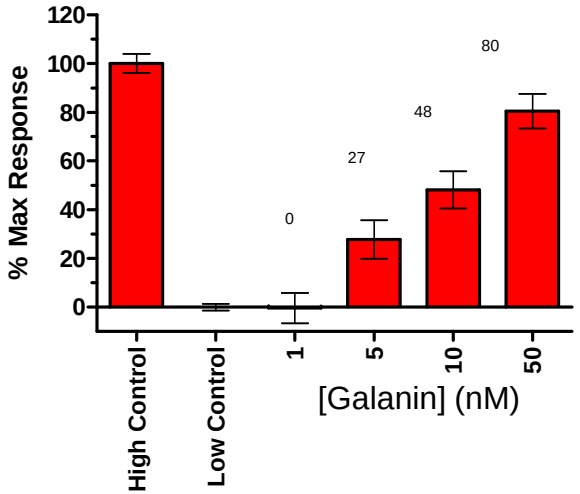
- Signal window is stable up to 24hrs
- Long read times (e.g. high plate numbers plates/reader delays) will not adversely affect assay performance



GalR2 IP1 HTS – Stability and variance of EC20

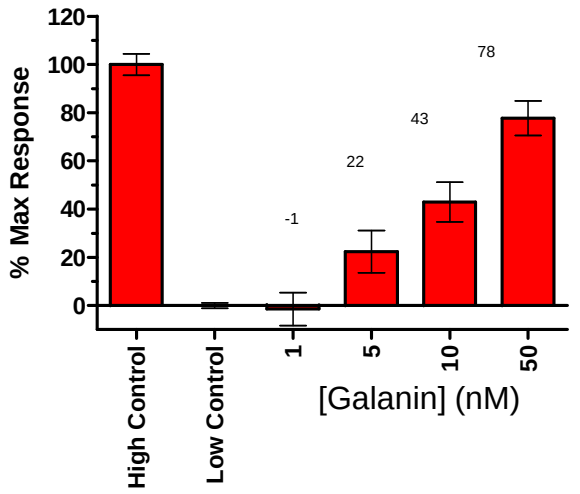
Day 1

Agonist-induced increase in [IP1] in CHO cells stably expressing GalR2 receptors



Day 2

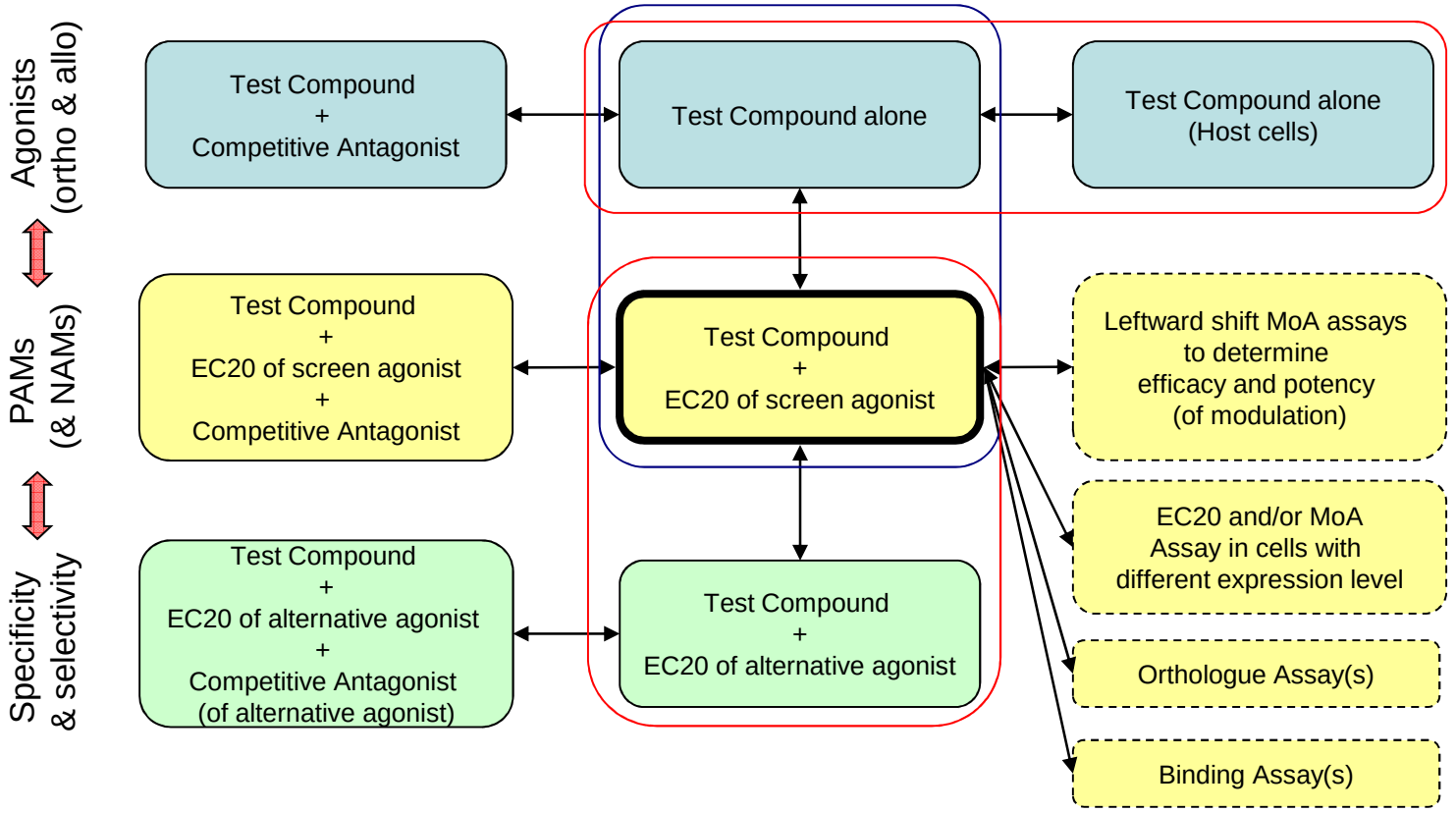
Agonist-induced increase in [IP1] in CHO cells stably expressing GalR2 receptors



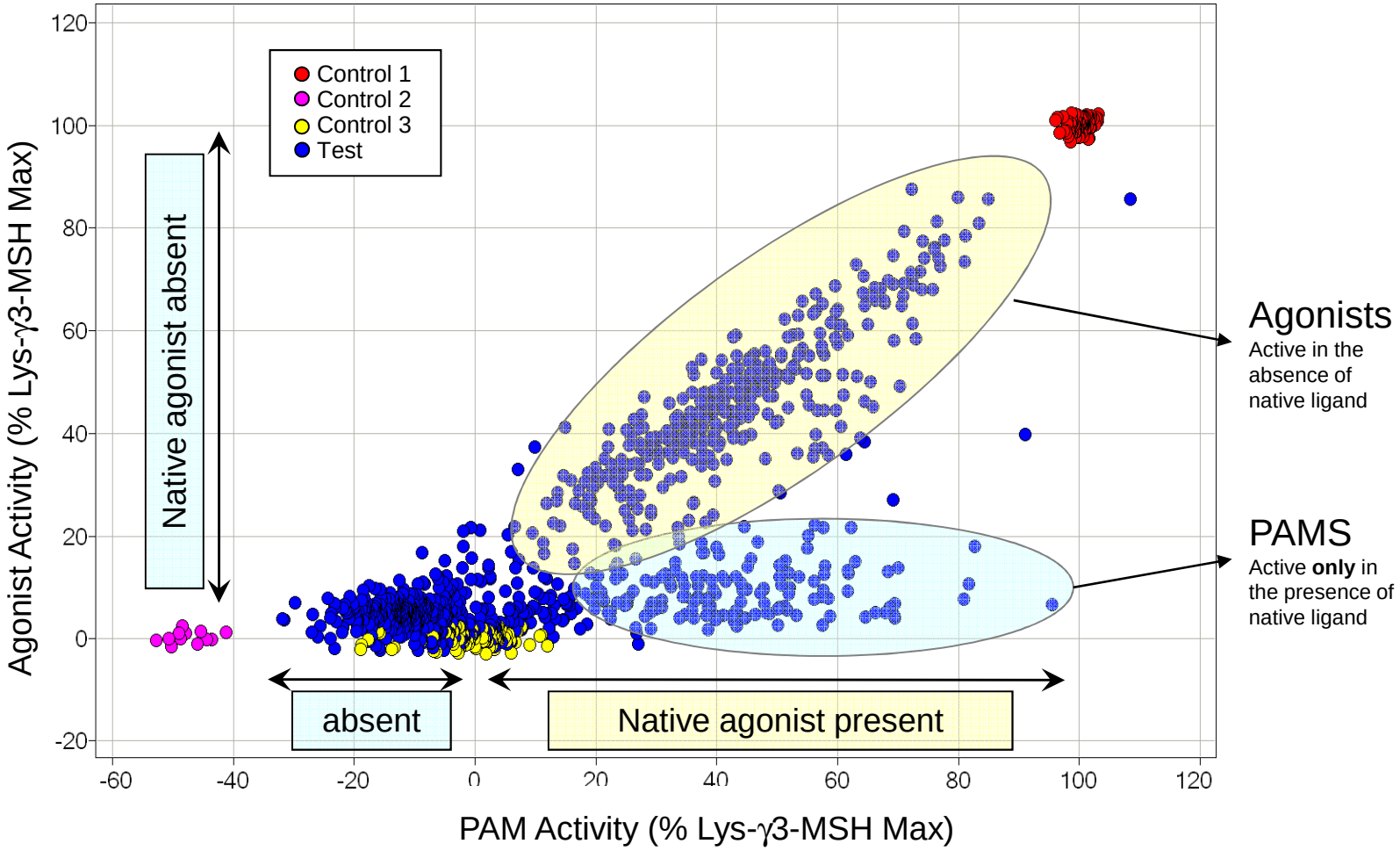
- 5nM Galanin produces a robust and stable response (~EC20)
- CVs <10%, window 2.5 fold, Z' 0.6 – 0.7

PAM HTS/CP – Major Challenges (2)

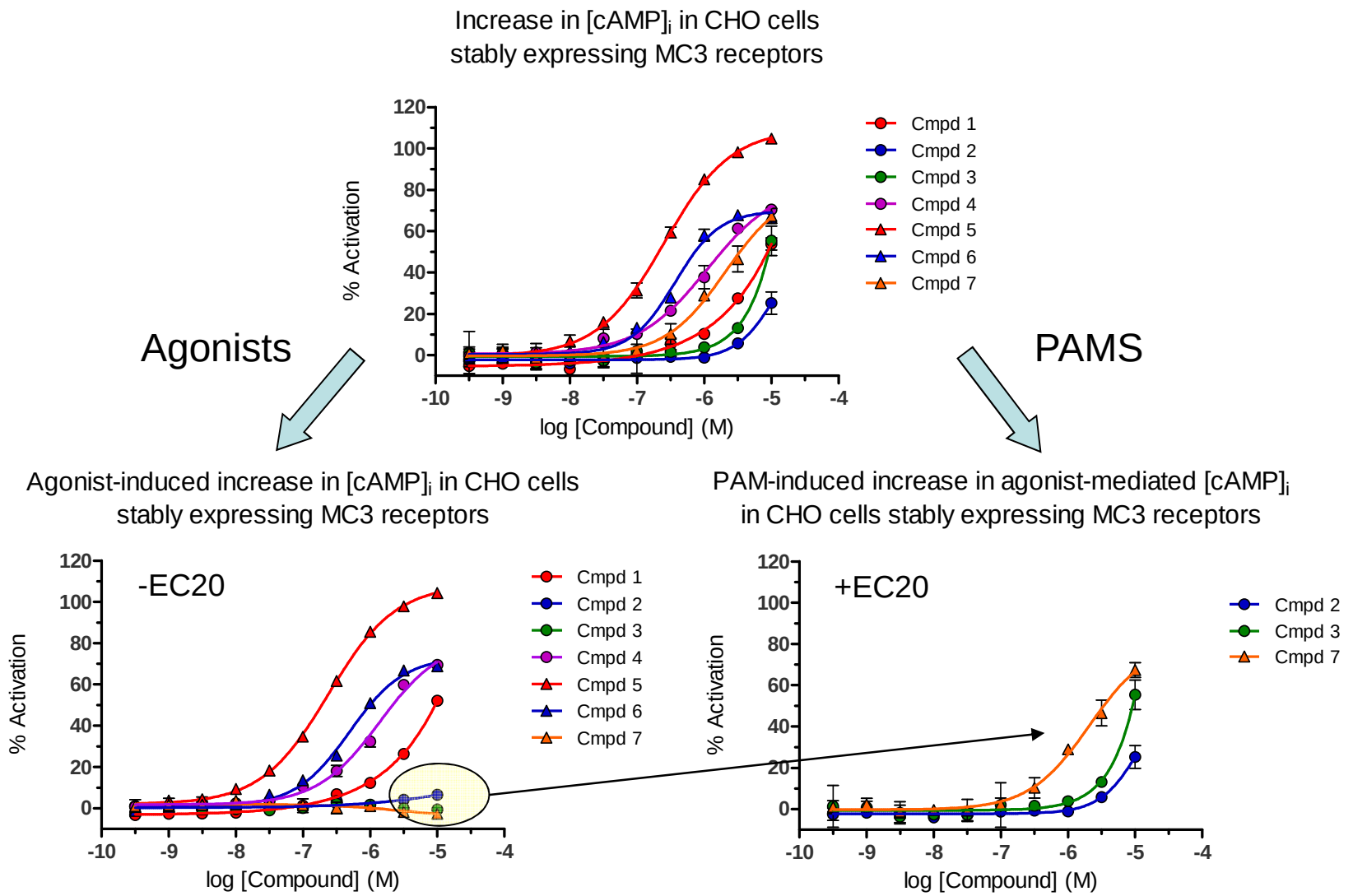
- Deconvolution of PAM/Agonist hits
- Removing false positives and/or non-preferred mechanism(s)



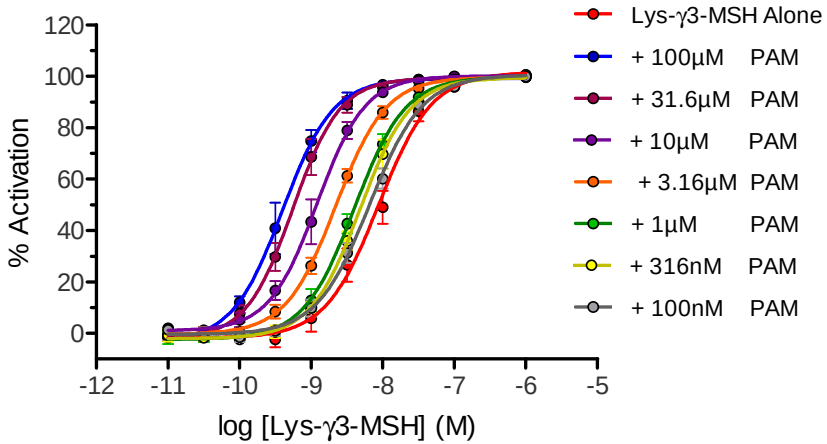
Deconvolution of PAM/Agonist modalities (Single Shot Triage)



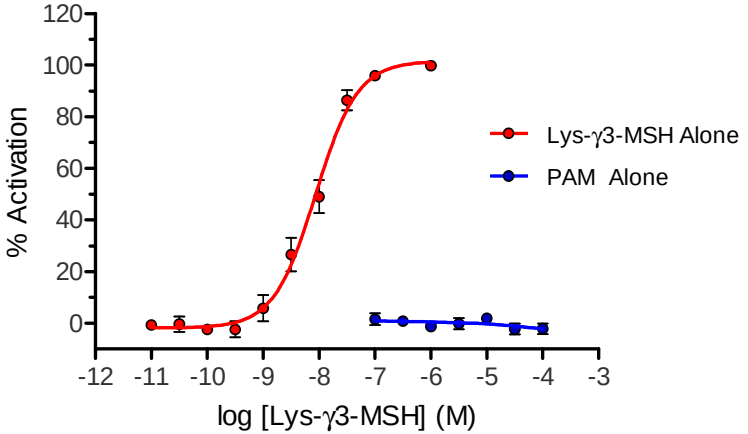
Deconvolution of PAM/Agonist modalities (Full Curve)



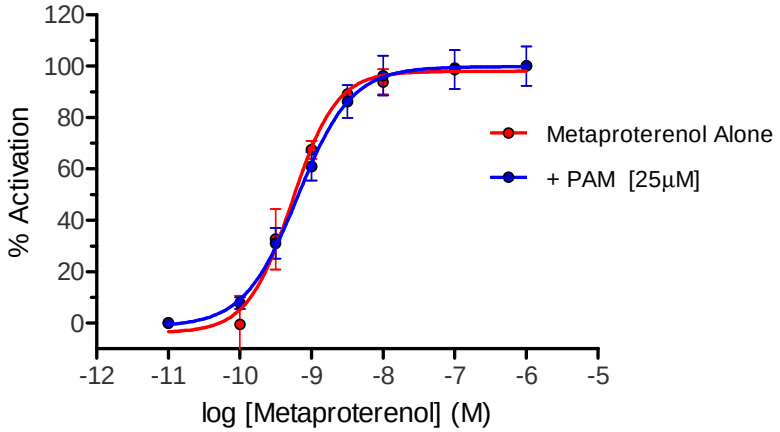
Agonist-induced increase in $[cAMP]_i$ in CHO cells stably expressing MC3 receptors (leftward shift)



Agonist-induced increase in $[cAMP]_i$ in CHO cells stably expressing MC3 receptors (leftward shift)



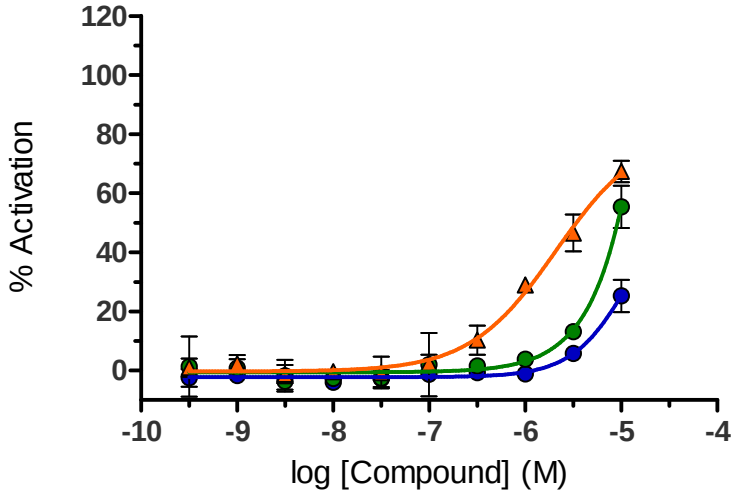
Agonist-induced increase in $[cAMP]_i$ in CHO cells stably expressing β_2 -Adrenoceptors (leftward shift)



- Shared robotic HTRF protocols facilitate;
  - *Assay in both single-point and full-curve mode (& transitions between)*
  - *Flexibility in assay design ($pEC50_{mod}$ vs leftward shift)*
  - *Simultaneous 'counter' assay (Ag vs PAM, off-target selectivity)*

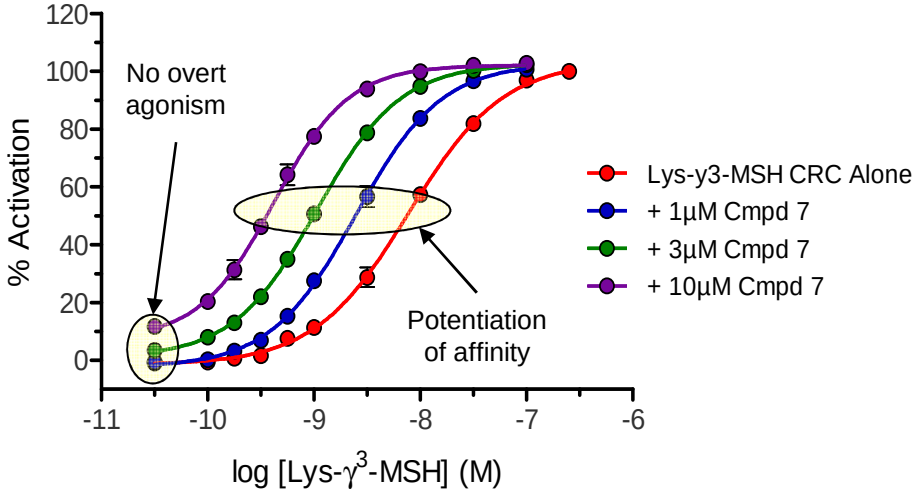
Apparent Potency in different assay formats

PAM-induced increase in agonist-mediated $[cAMP]_i$
in CHO cells stably expressing MC3 receptors



Moderate pEC50 of modulation (EC20 mode)
translates to very effective leftward shift

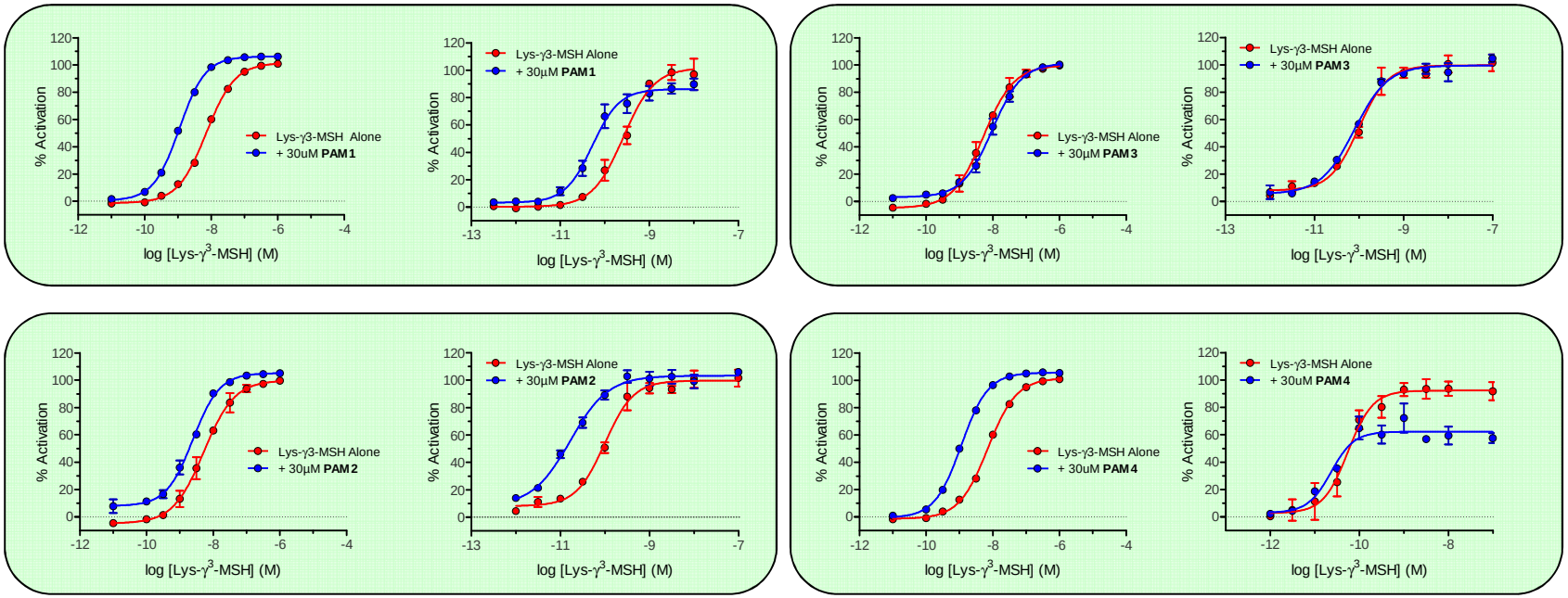
Agonist-induced increase in $[cAMP]_i$ in CHO cells
stably expressing MC3 receptors
(leftward shift)



PAM HTS/CP – Major Challenges (3)

- *Differences* in pharmacology between cAMP detection systems
  - *PAM 'activity' may not necessarily align between detection formats*

HTRF *CRE HTRF *CRE



*GeneBLAzer; β-lactamase coupled to a cyclic AMP response element (CRE)

Summary

- HTRF provides a sensitive and stable assay from which to configure PAM assay(s)
  - *In HTS mode the stability of EC20 is pivotal to PAM sensitivity*
- HTS and full curve hit profiling assays can be configured to share common (simple) robotised protocols
  - *pEC50 of modulation (underpinned by EC20)*
  - *Partial or full leftward shifts provide texture to PAM activity*
  - *Quantitative pharmacological analyses e.g. ETCM modelling to dissect potentiation of affinity vs efficacy*
- The technology lends itself to establishing appropriate and necessarily extensive deconvolution assays
  - *Which can share a common detection platform*

Summary

- The accumulation nature of the signal affords greater flexibility in compound pre-exposure
  - *Arguably improving the sensitivity to slow binders (PAMS)*
  - *Circumventing confounding kinetic issues with more transient detection systems (Ca^{2+})*
- The pharmacology of PAMs is complex and 'perfect' alignment with other (cAMP) detection technologies is likely to be rare
  - *A plethora of biological factors can give rise to subtle differences in apparent PAM pharmacology*

Acknowledgments

MRC Centre for Therapeutics Discovery

- Ahmad Kamal
- Jenny Cook
- Rachel Forfar
- Debra Taylor
- Hayley Jones
- Paul Wright
- Puneet Khurana

University of Bristol

- David Wynick

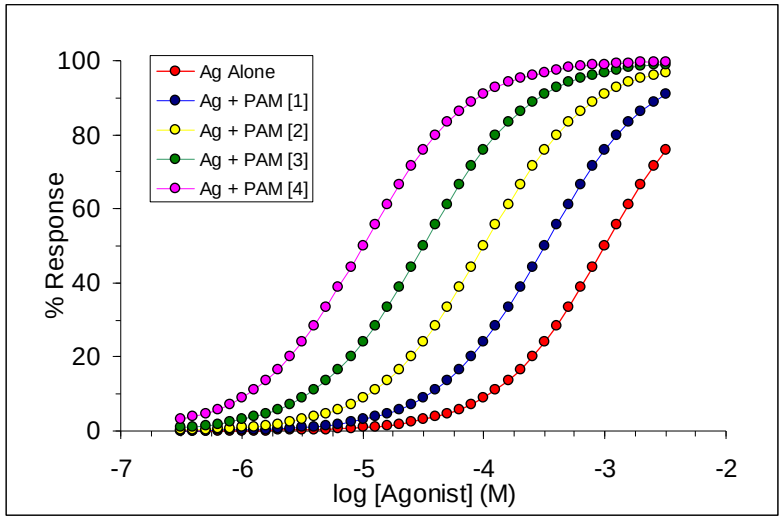
Queen Mary University of London

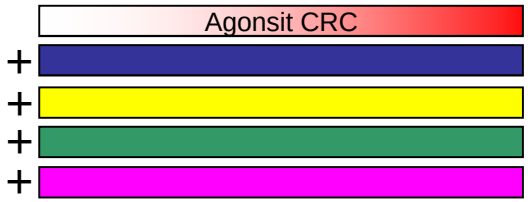
William Harvey Research Institute, Queen Mary University

- Mauro Perretti
- Trinidad Montero-Melendez

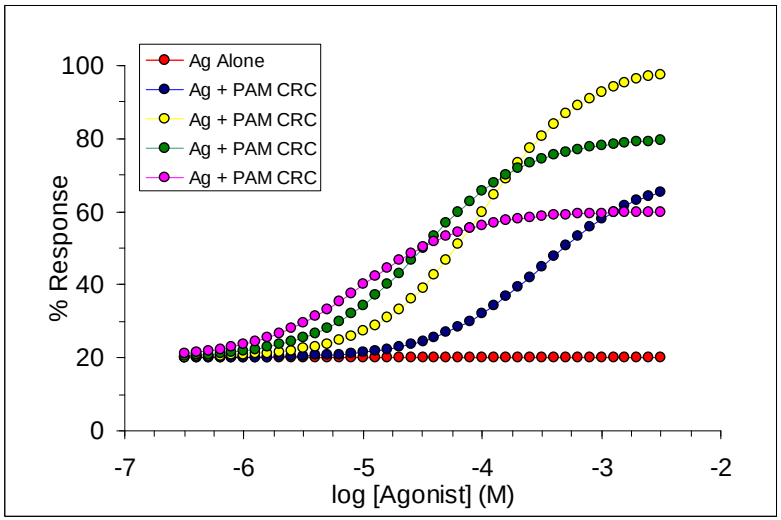
Alternative PAM assay configurations

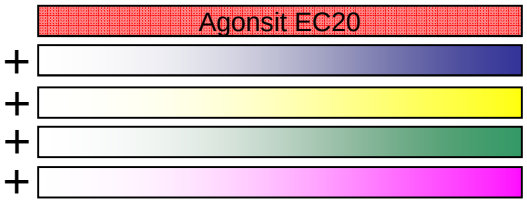
‘Leftward Shift’



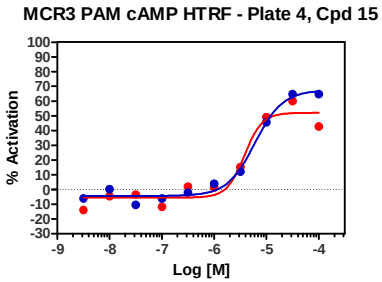
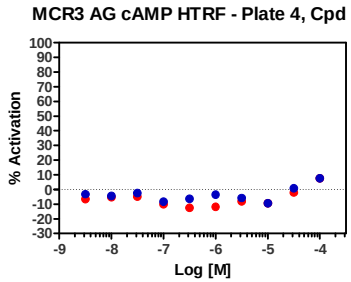
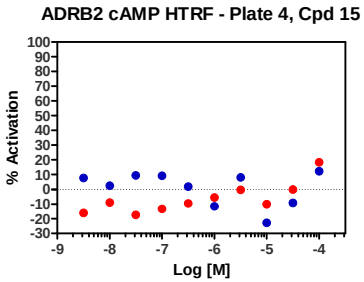
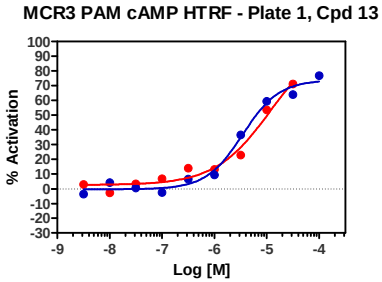
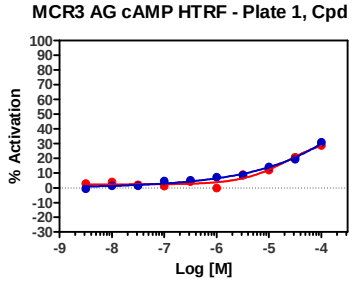
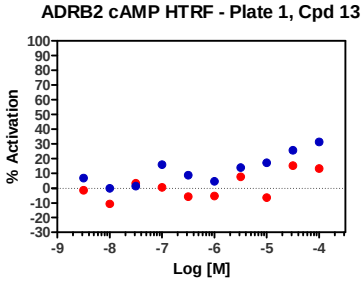


‘EC20’





Exemplar curve signatures

	PAM MC3	Ag MC3	PAM β2
‘Clean’ PAM			
PAM with (allo) agonism			
Non specific compound (receptor and or mechanism)