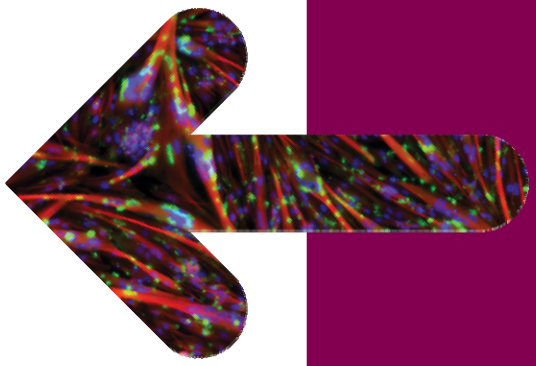
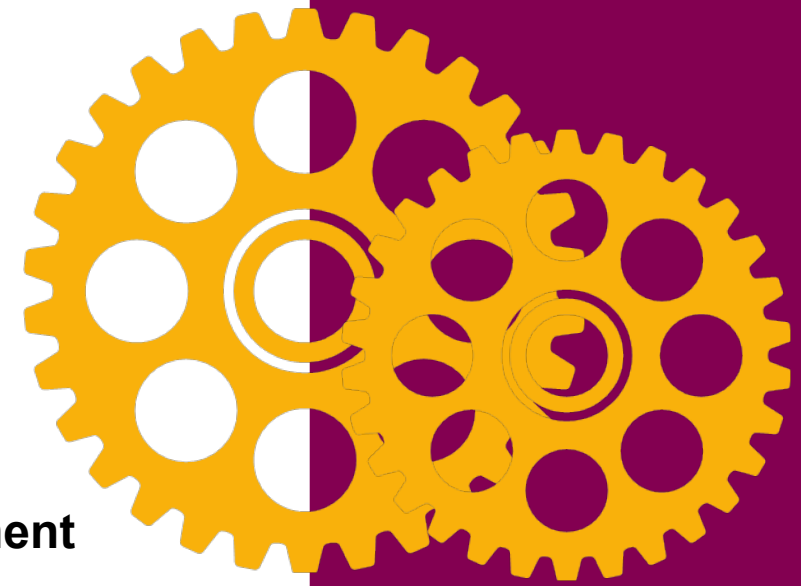
The challenges facing PI3K in drug discovery from an in vitro assay and screening perspective.





ELRIG Drug Discovery
September 2013

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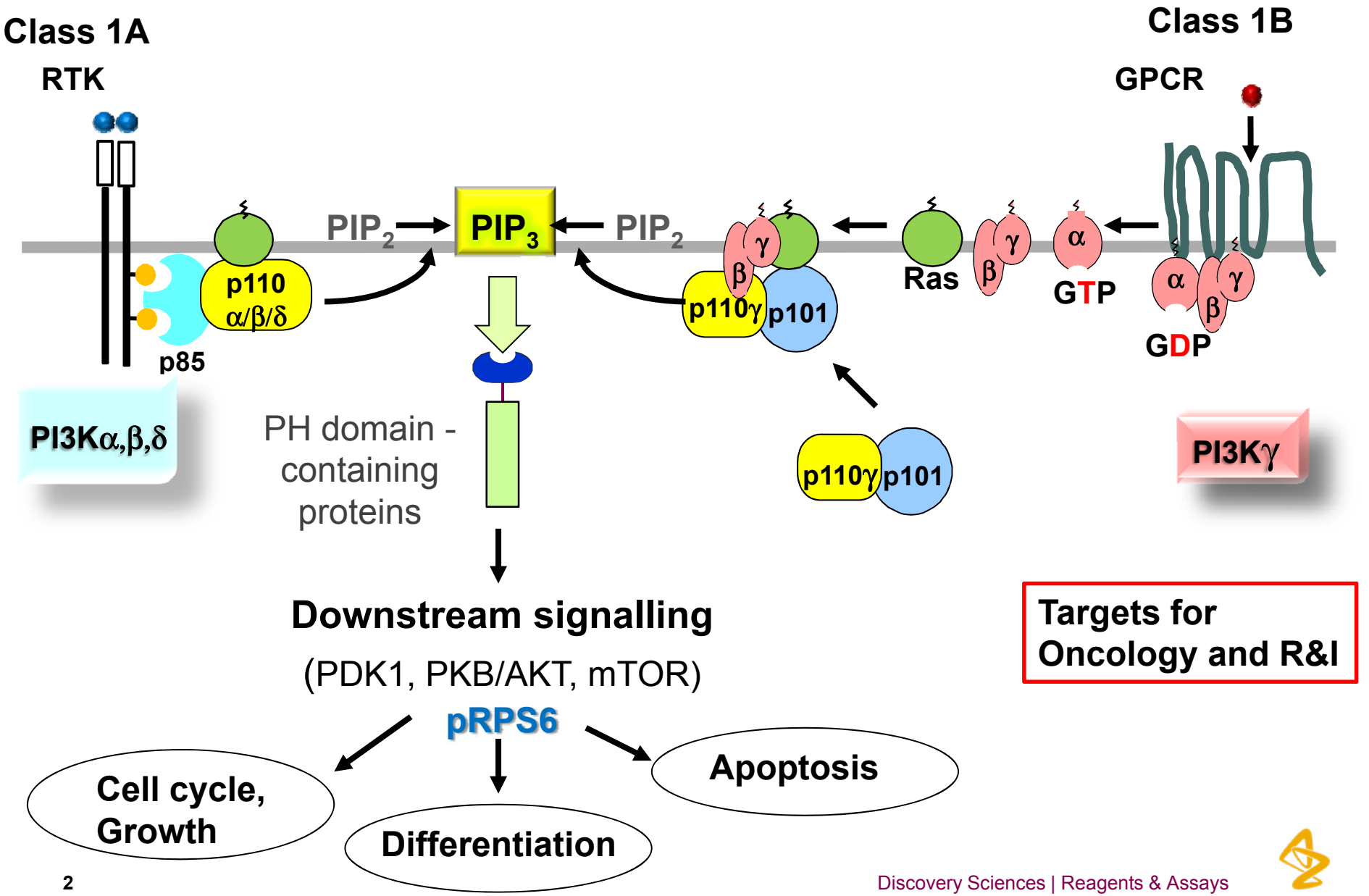
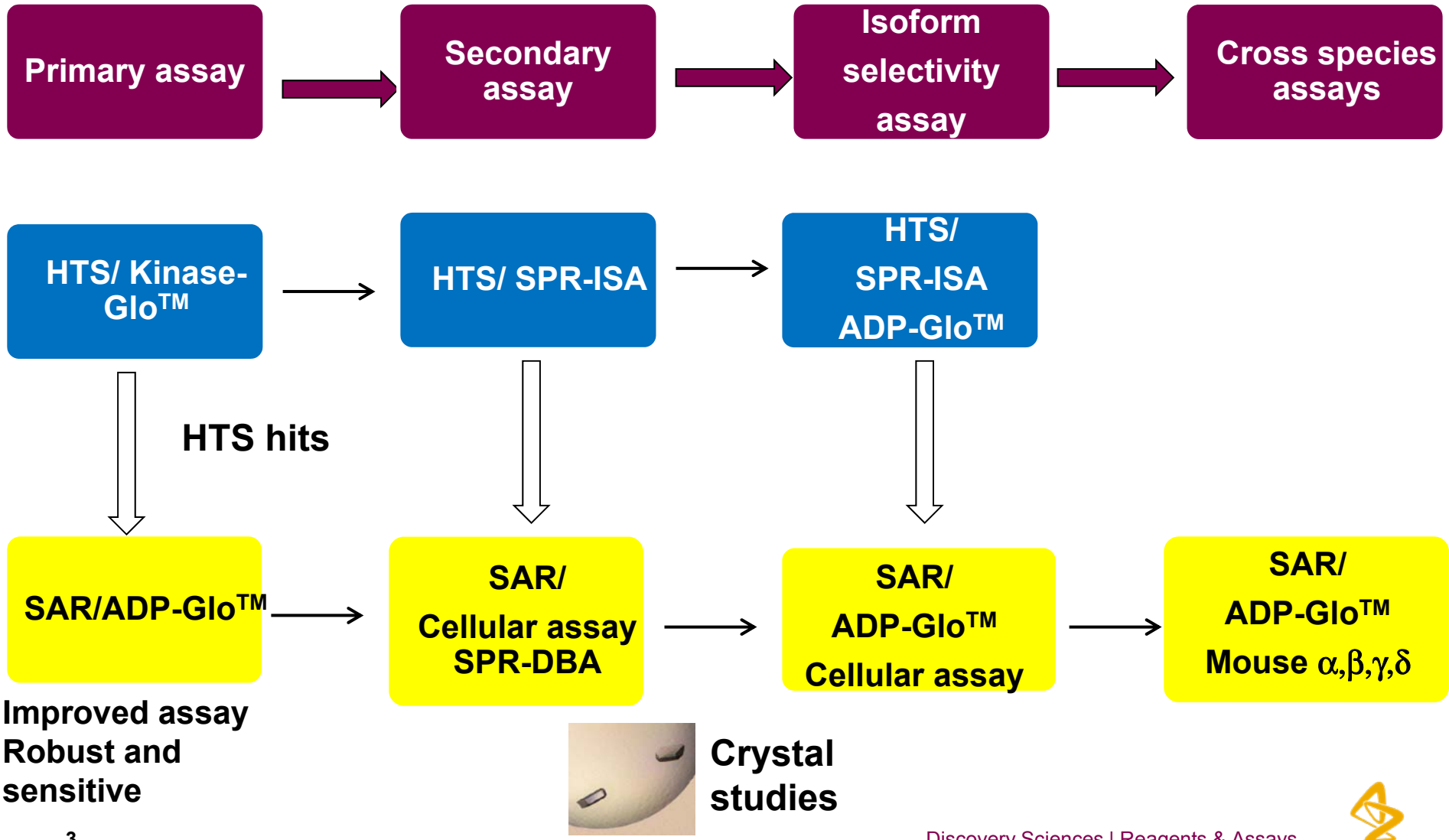
Reagents and Assay Development
Discovery Sciences
Mölndal

AstraZeneca

Class I PI3K cellular signalling

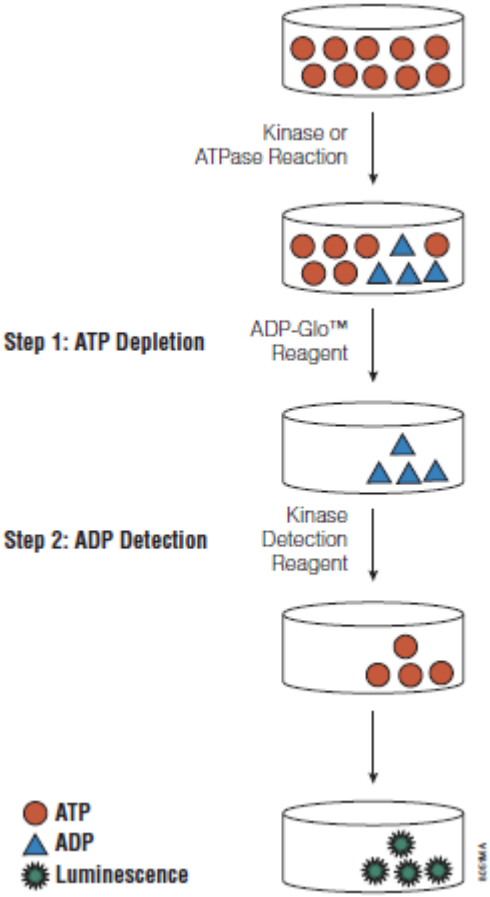


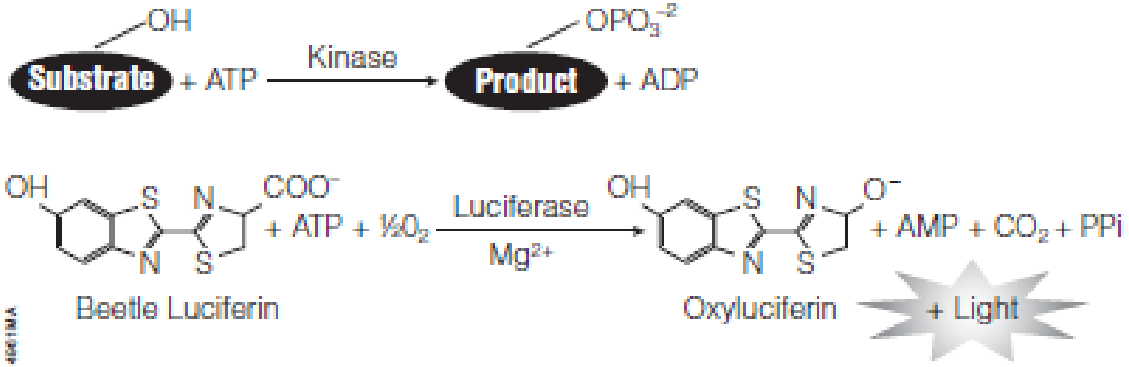
In Vitro Assay Screen Cascade



Primary assay

Assay Principle Kinase-Glo™ – ADP-Glo™





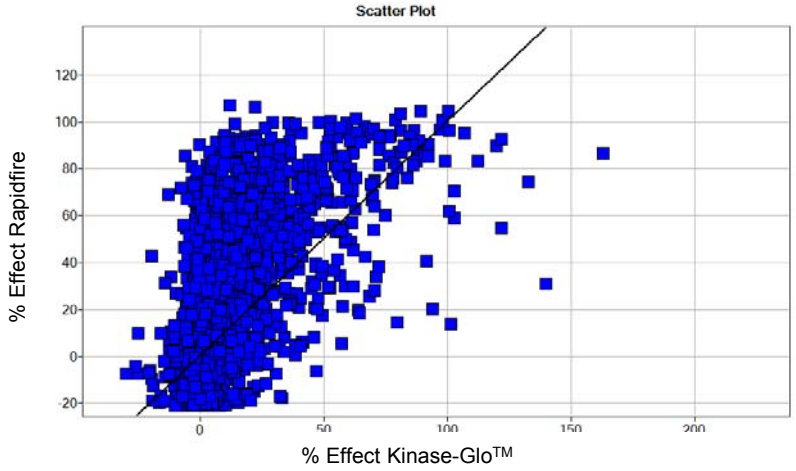
ADP-Glo™

improved assay for all PI3K isoforms

- ✓ stable signal at higher ATP conc.
- ✓ assay stable at lower enzyme conc.
- ✓ statistically more robust for SAR

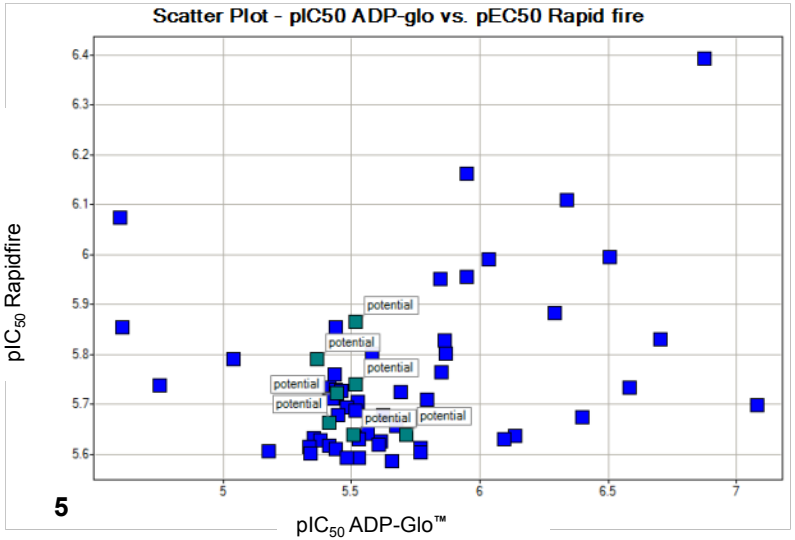
Parallel assessment of assays for Primary screen

Kinase-Glo™ vs Rapidfire – 10K subset screen



Correlation evident but overlap of compounds designate as hits rather low (Around 30%).
70% of compounds designated as hits in Rapidfire were not picked up in Kinase-Glo™.

ADP-Glo™ vs Rapidfire - Dose response



60 compounds active from Rapidfire in a pIC_{50} range of 5.5-6.5 and not picked up by Kinase-Glo™ were run as dose response in ADP-Glo™ and all came back as active.

→ ADP-Glo™ more sensitive than Kinase-Glo™
→ ADP-Glo™ acceptable for SAR screening

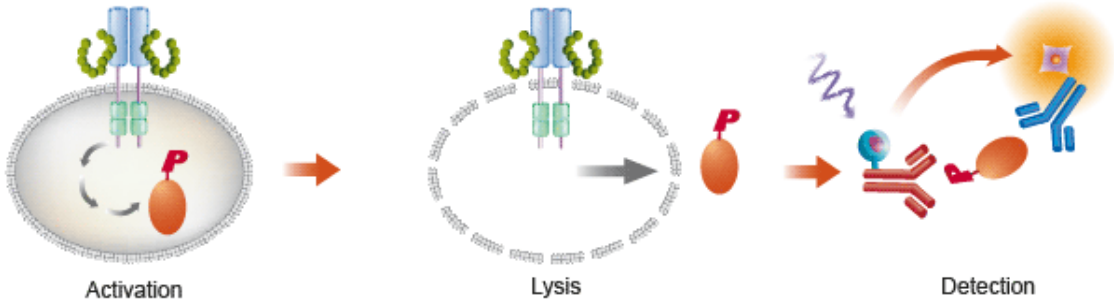
Cellular assays-

functional assay with inhibition of phosphorylated AKT (Ser473)

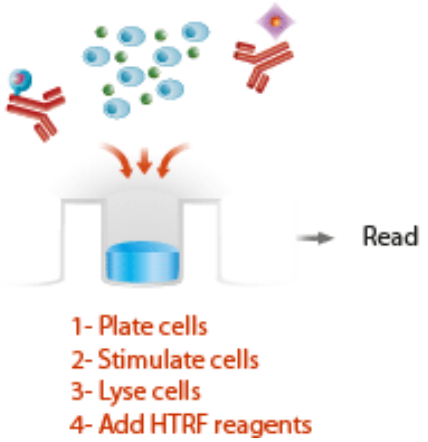
- PI3Kδ – human permanent B cell line – Jeko-1. pAKT stimulation with Anti IgM via the B cell receptor
- PI3Kγ – mouse monocytic cell line – RAW264. pAKT stimulation with C5a via the GPCR signalling

ATP conc. in cells in mM range.

Assay layout - HTRF™

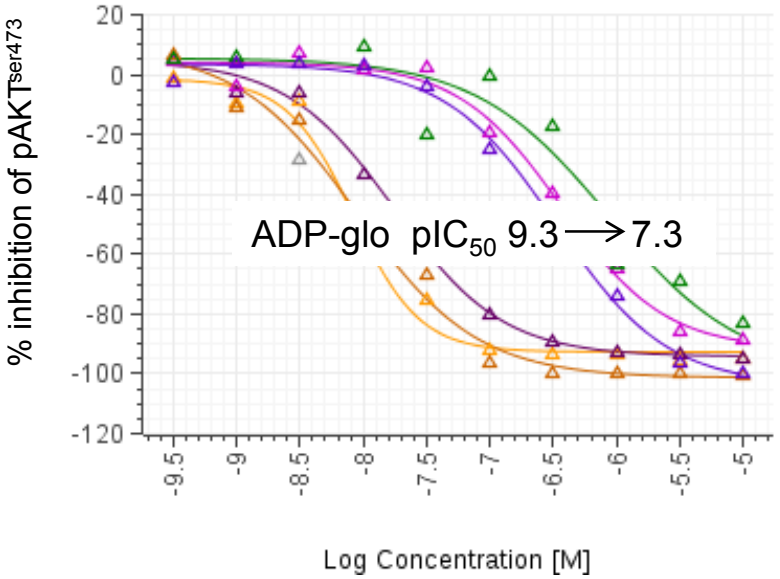


One step assay in 384 plates.



Translation of compounds into cellular assay

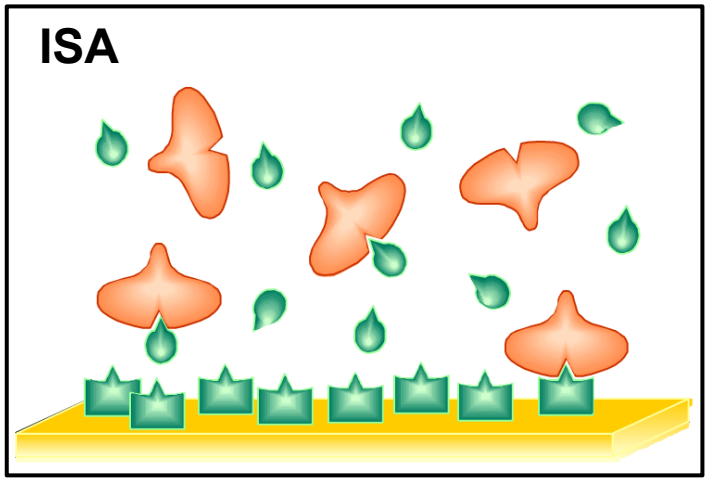
RAW264 cells stimulated with C5a



Compound	ADP-glo™ γ pIC_{50}	pAKT γ pIC_{50}	ADP-glo™ δ pIC_{50}	pAKT δ pIC_{50}
IC87114	6.0 (5.3)	5.0	7.3 (6.9)	7.3 (++)
AS252424	7.6 (7.9)	5.7 (6.0)	7.0 (6.7)	5.0
IPI145	8.2 (7.4)	6.9 (7.3)	9.5 (8.6)	9.4 (9.0)
GSK2269557	5.8 (6.0)	5.1	9.4 (9.8)	9.5 (9.6)

(Published data in brackets)

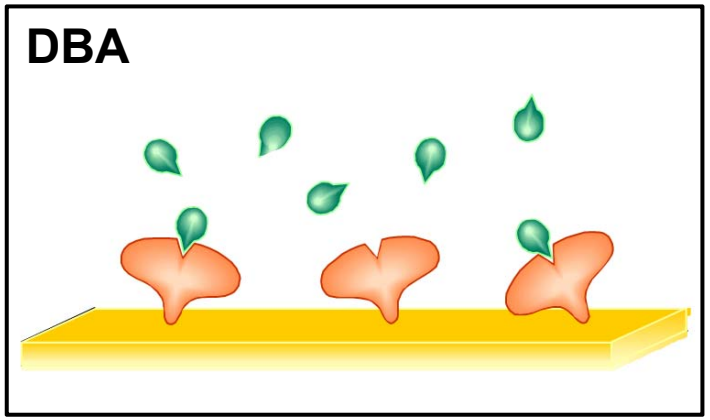
Assay formats on Biacore/SPR biosensors



= Target definition compound (TDC)

= Compound/Fragment

= Protein



Inhibition in solution assay (ISA)

- Interaction of protein and compound occurs free in solution
- K_D determinations, but no kinetic information
- Requires immobilisation of a Target definition compound (TDC)
- **Used for target engagement and isoform selectivity**

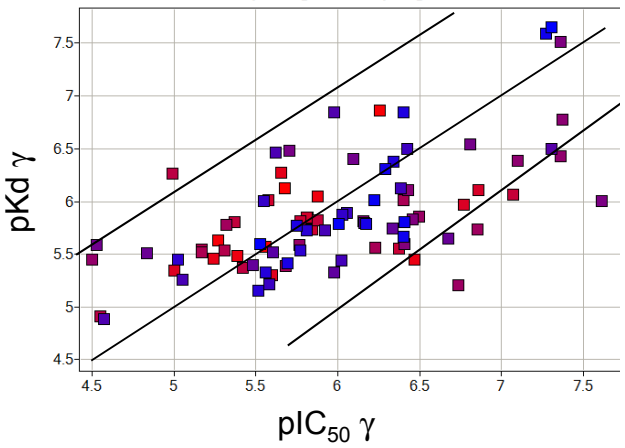
Direct binding assay (DBA)

- Protein immobilisation and reuse of same surface
- Determination of affinity and kinetic
- **Used for determination of sub-nM potencies and MOA**

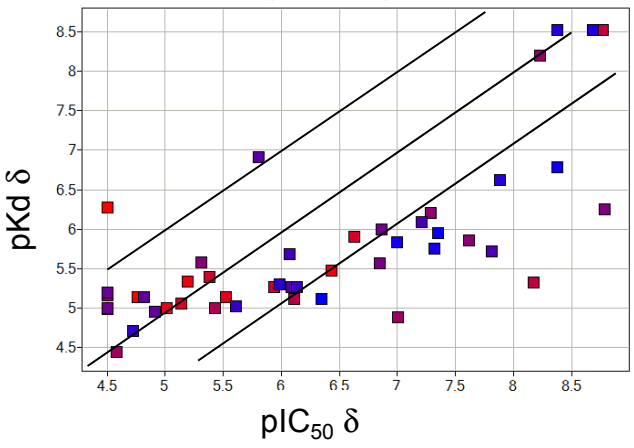
Inhibition in solution assay – ISA

Follow up on HTS hits

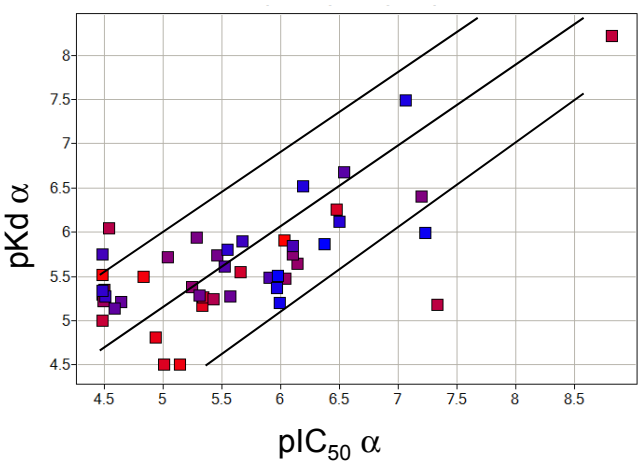
PI3Kγ



PI3Kδ



PI3Kα



Achievements;

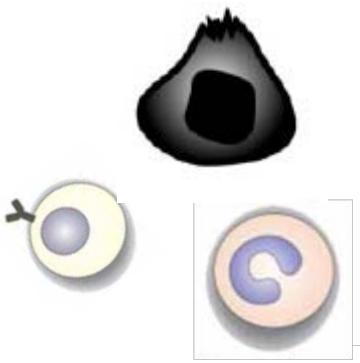
- Hits from ADP-Glo™ were confirmed as binders in SPR.
- Selectivity confirmed between isoforms.

Learnings;

- The precision in ISA can not be compared with DBA.
- Tricky to find the right TDC.

Isoform selectivity assays PI3K;

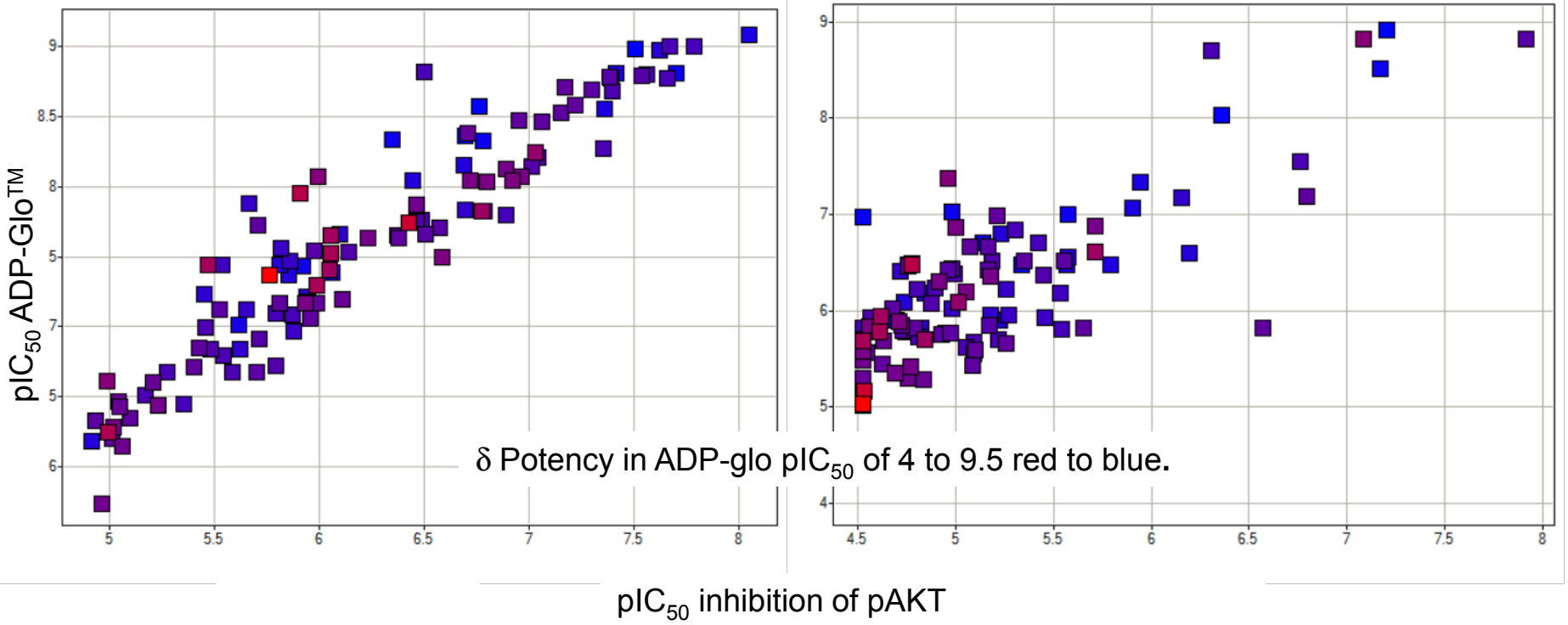
Biochemical versus cellular assay



Divers origin of cell lines used in assay

PI3K α

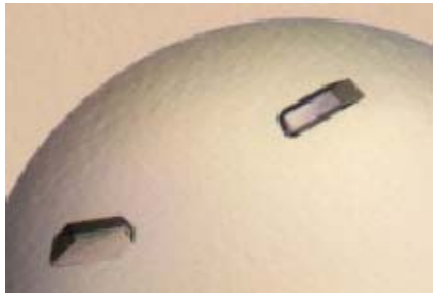
PI3K β



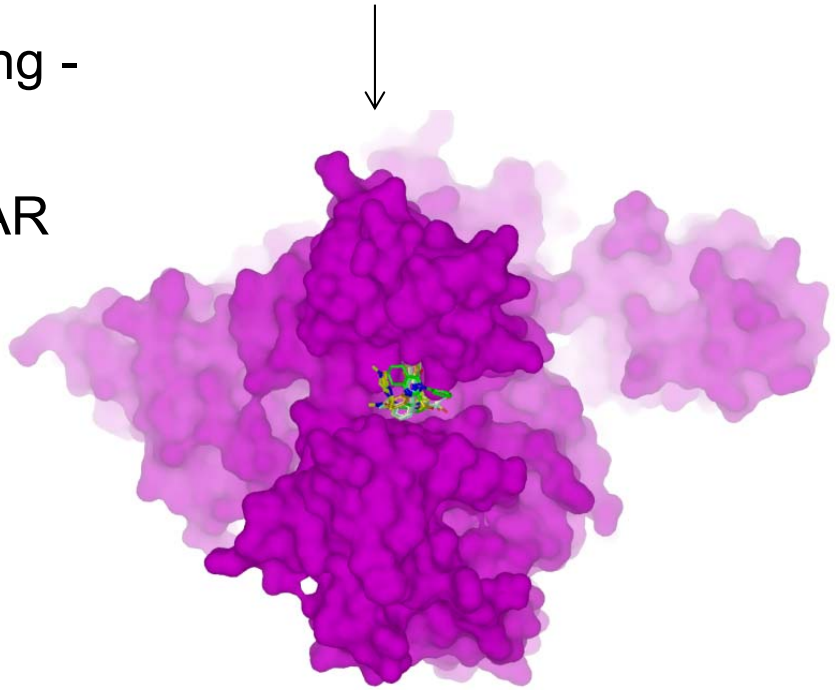
Biochemical assay used for selectivity between PI3K isoforms

Crystallography
Binding Modes & Selectivity

Ligand co-crystal structures are delivered in a iterative system for PI3Kγ,δ



- ✓provide information on differences in binding - mode between isoforms
  - Selectivity
- ✓maps out active site to help understand SAR
  - ✓ Potency
- ✓drive design ideas
  - ✓ Chemical diversity and novelty



In vitro screening cascade established

Major challenges and solutions;

- **Tight binders (nM to sub-nM potencies);**
  - ✓improved primary assay - robust at low enzyme conc.
  - ✓SPR-DBA
- **Secondary assay;**
  - ✓homogeneous cellular assays with a good through put
  - ✓SPR – ISA and DBA
- **Selectivity between PI3K isoforms;**
  - ✓biochemical and cellular assays – relevant to understand selectivity in target cells
  - ✓crystal structures – differences in binding mode
  - ✓broader kinase selectivity strategy – external partners

Acknowledgements;

Discovery Sciences

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