

Use of
Label-Free Detection Technologies
in the
Hit-to-Lead Process

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Acknowledgements

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Outline

Overview Label Free Technologies

Evaluation Corning Epic System

Integration into Drug Discovery Process

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Integration into Drug Discovery Process

Label Free Technologies Overview

Biochemical Assays

Mechanical

Acoustic

Calorimetric

SPR

Ion Channels

HT Electrophysiology

Other

Cellular Assays (GPCR)

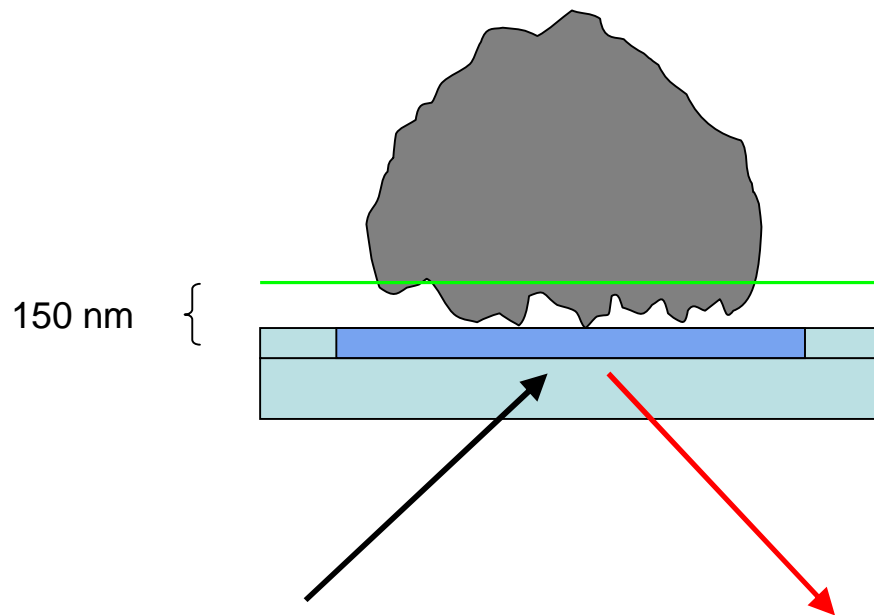
Impedance *

- Acea (Roche)
- Cellkey (MDS)

Surface Optical *#

- Epic (Corning)
- BIND (SRU Biosystems)

Physical Principle Surface Optical Epic Platform



Sensor (with waveguide grating) is illuminated with broadband light source

Wavelength resonant with waveguide grating is reflected

Change of index of refraction at surface causes **shift of resonant wavelength** (need to match DMSO)

Measurement relative to baseline

Morphological Changes

Dynamic Mass Redistribution

* **Horizontal**

* **Vertical**

Corning Epic Equipment: Assay Workflow



Cell based assays

1. Plate cells (fresh or frozen) at different seeding densities (around 8 to 16k cells / well)
2. Allow cells to adhere to plate (overnight).
3. Remove assay medium and replace with assay buffer.
4. Allow cells to equilibrate in the Epic instrument at 26C.
5. Perform baseline measurement.
6. Add compounds
7. Read immediately
8. Add agonist (or compound)
9. Read immediately

Main factors affecting assay quality:

Serum concentration

Cell number

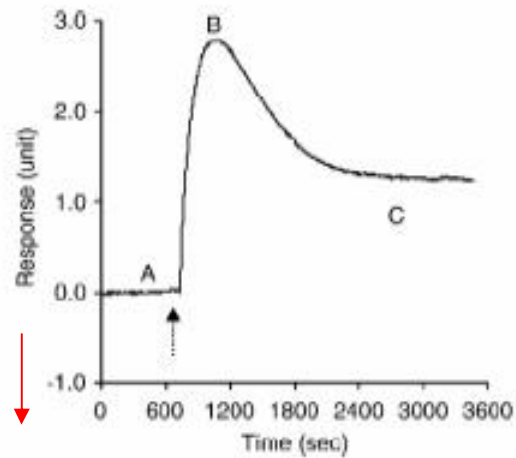
Buffer exchange

Equilibration- and measurement time

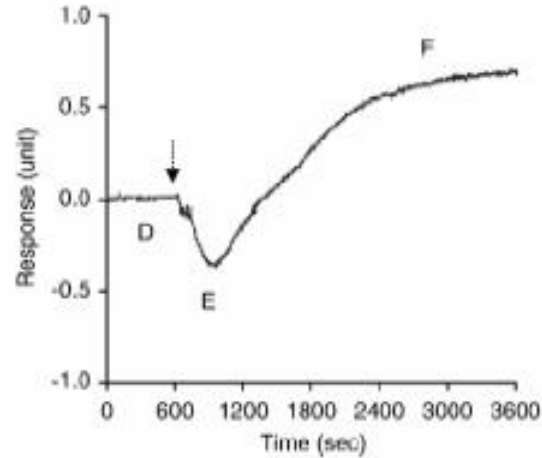
Integrated liquid handling recommended

Epic Signal Pattern

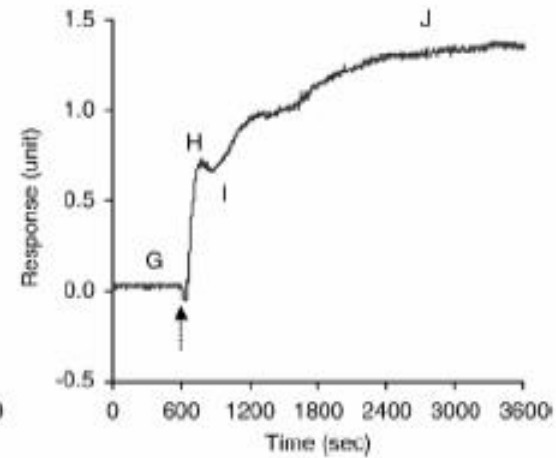
Gq



Gs



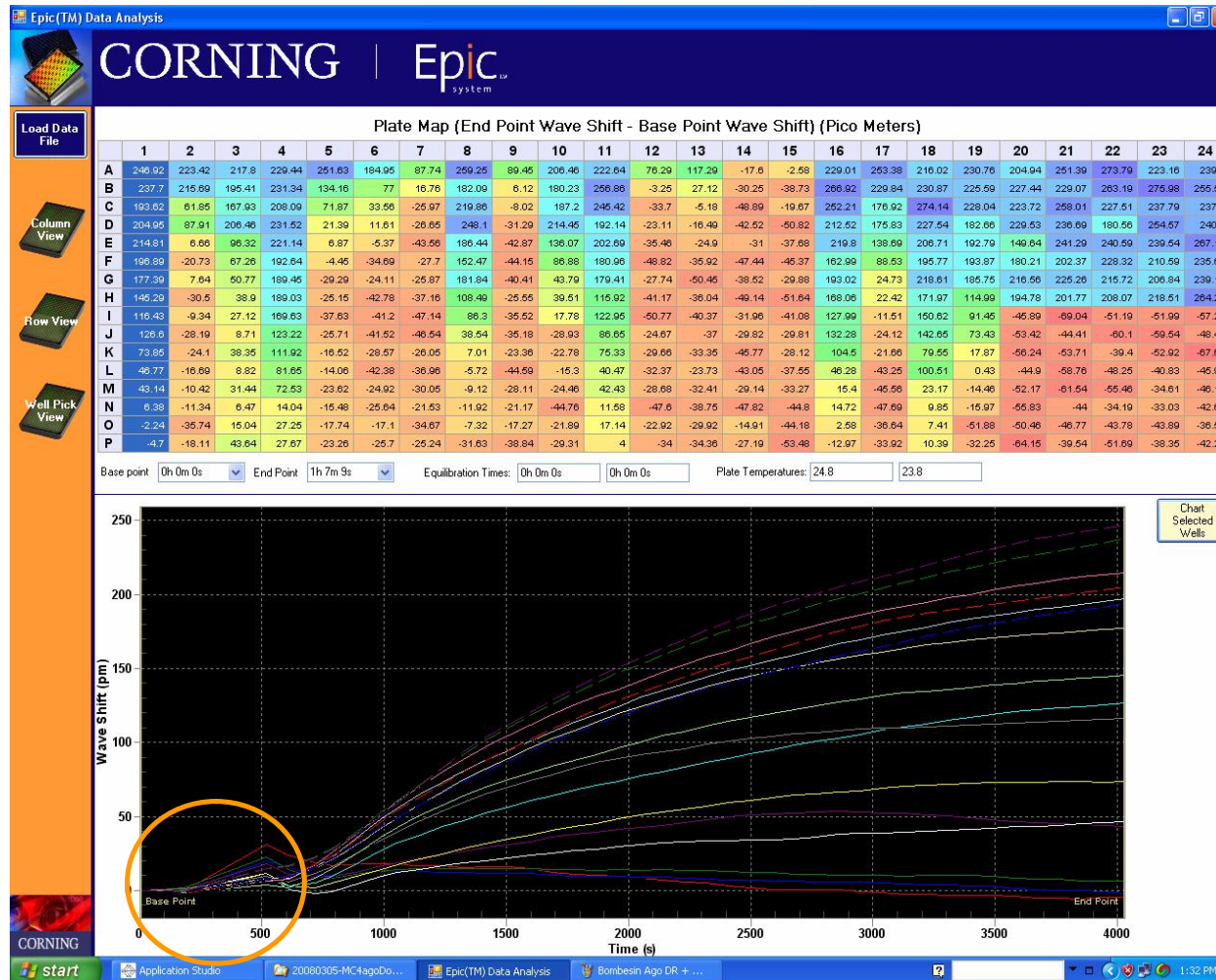
Gi



1 unit
=
100 pm

Signal patterns can be more complex (here: A431 background)

Epic Data Agonist (G_s coupled GPCR)



Outline

Overview Label Free Technologies

Evaluation Corning Epic System
Focus on GPCRs

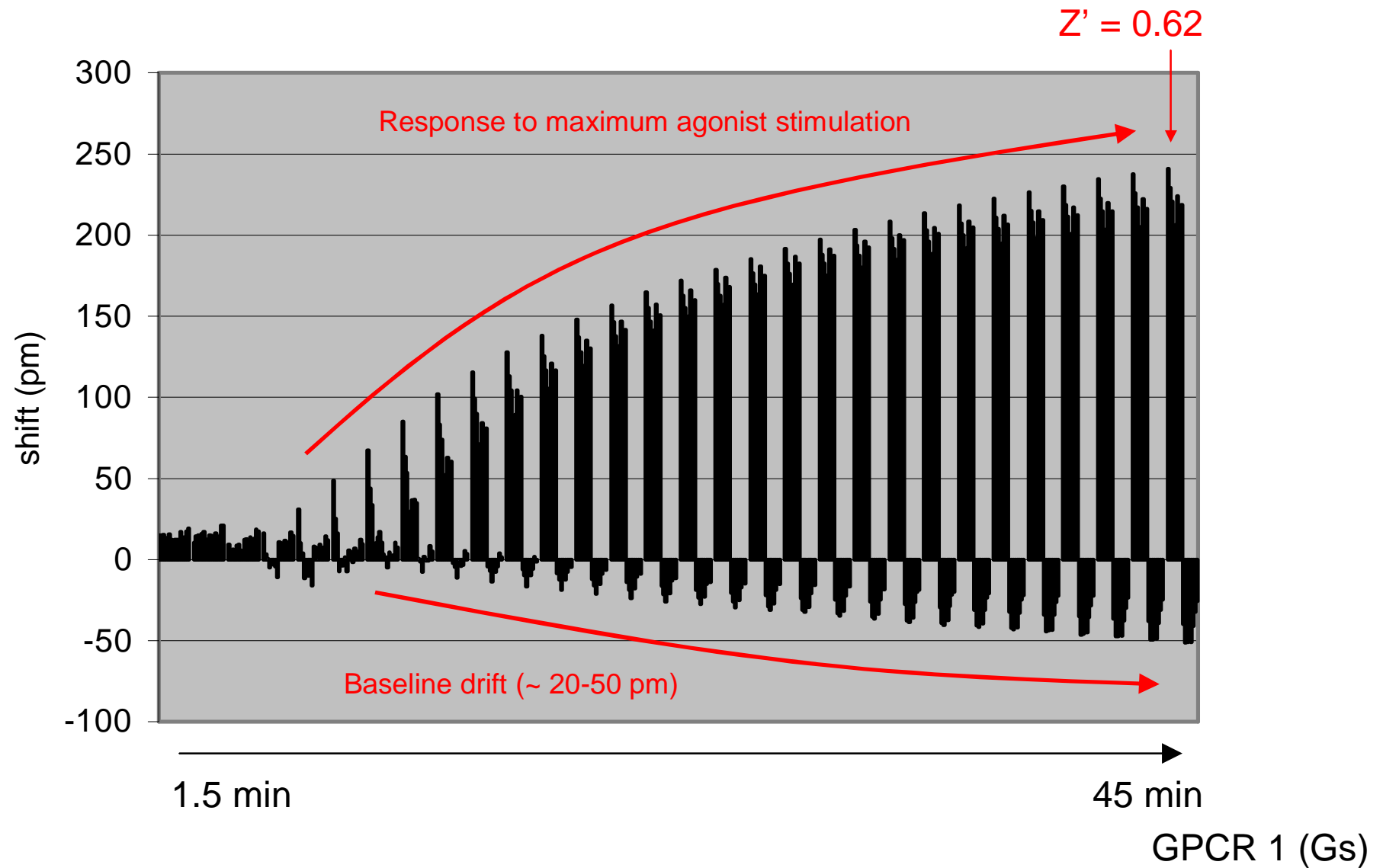
Integration into Drug Discovery Process

Epic evaluation trial

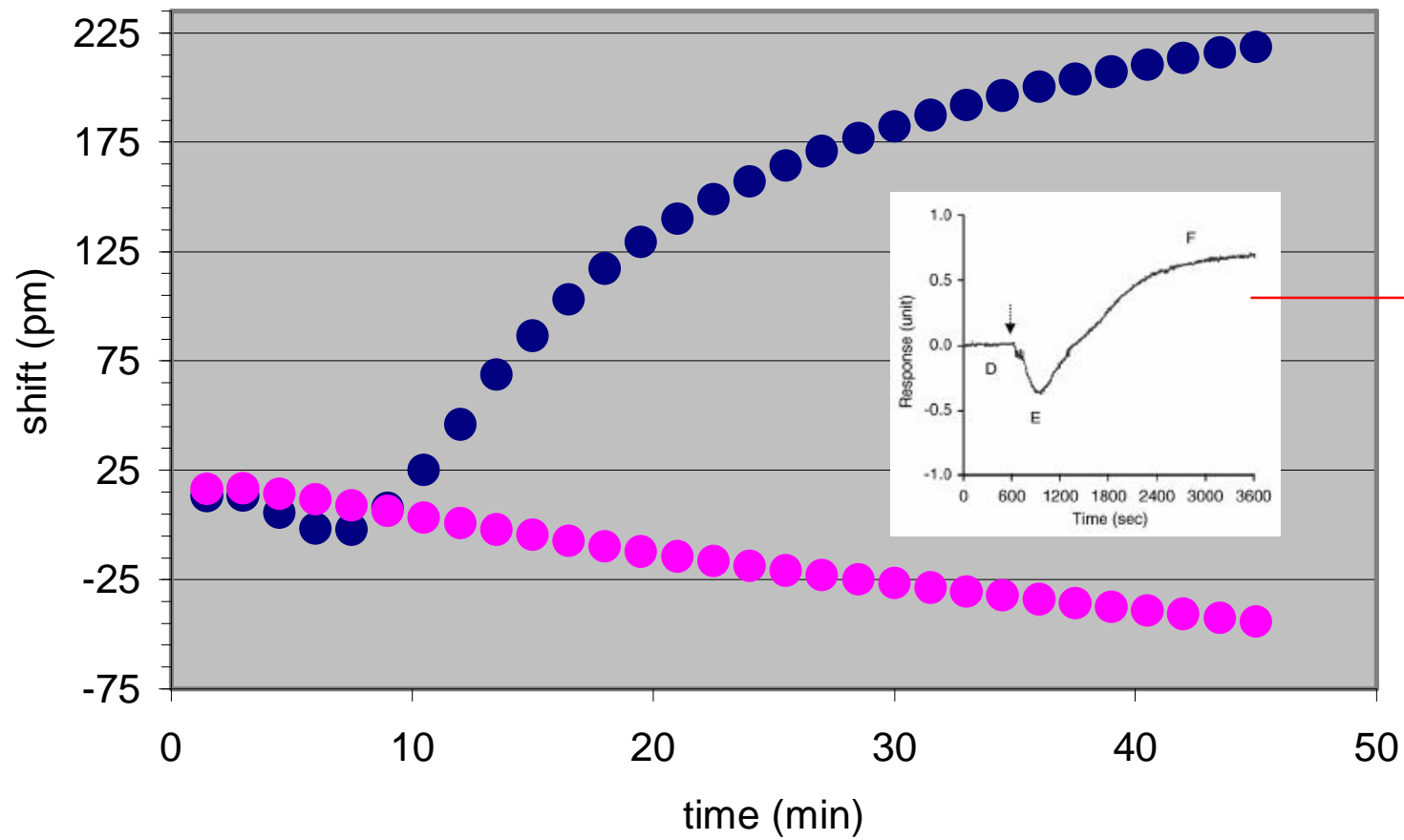
- Goals:
 - Understand overall performance of system
- Focus on a panel of (recombinant) GPCRs
 - Shotgun approach
 - Some systems investigated in more detail
- 16 different recombinant cell lines (GPCRs)
 - Biochemical assay (Iva Navratilova) *
- No experience under true HTS conditions
 - Only small number of AVR plates
- Some (limited) experience with primary cells *

* not covered in this presentation

G_s Coupled Peptide GPCR Raw Data



G_s GPCR Signal Pattern

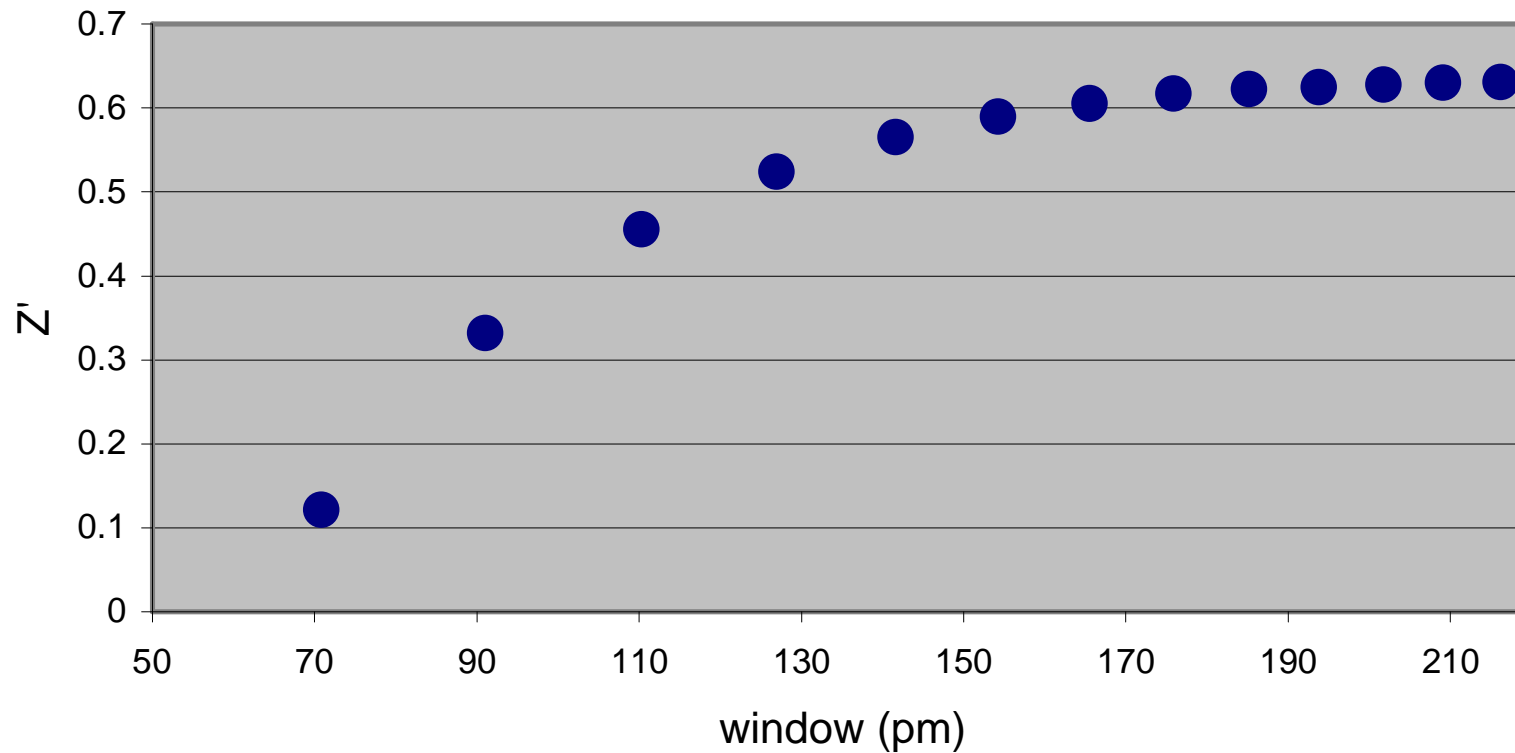


GPCR 1 (G_s)

100 -150 pm window sufficient

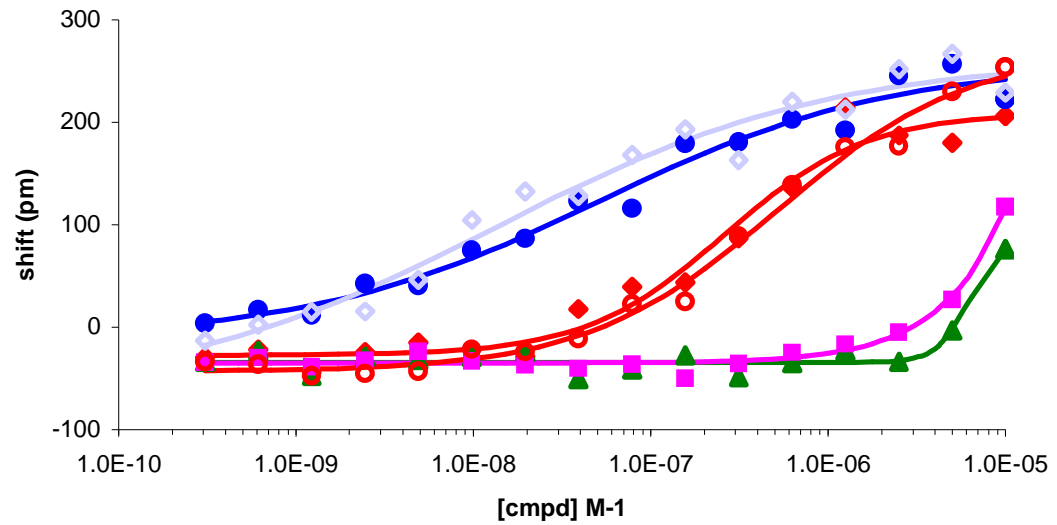
Single Point Assays

Z' vs. assay window Gs coupled GPCR

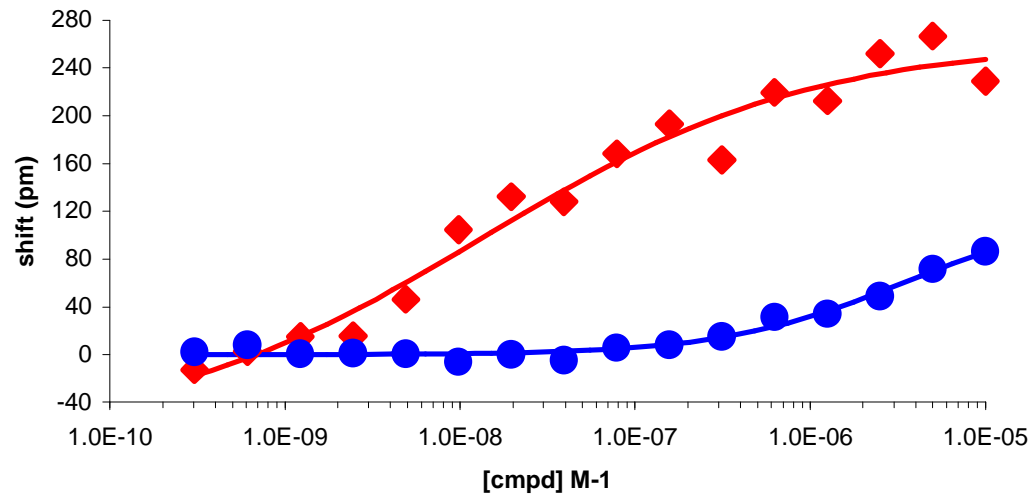


GPCR 1 (Gs)

Gs coupled peptide GPCR selected dose response
(end of measurement)



Gs coupled peptide GPCR time dependence EC50



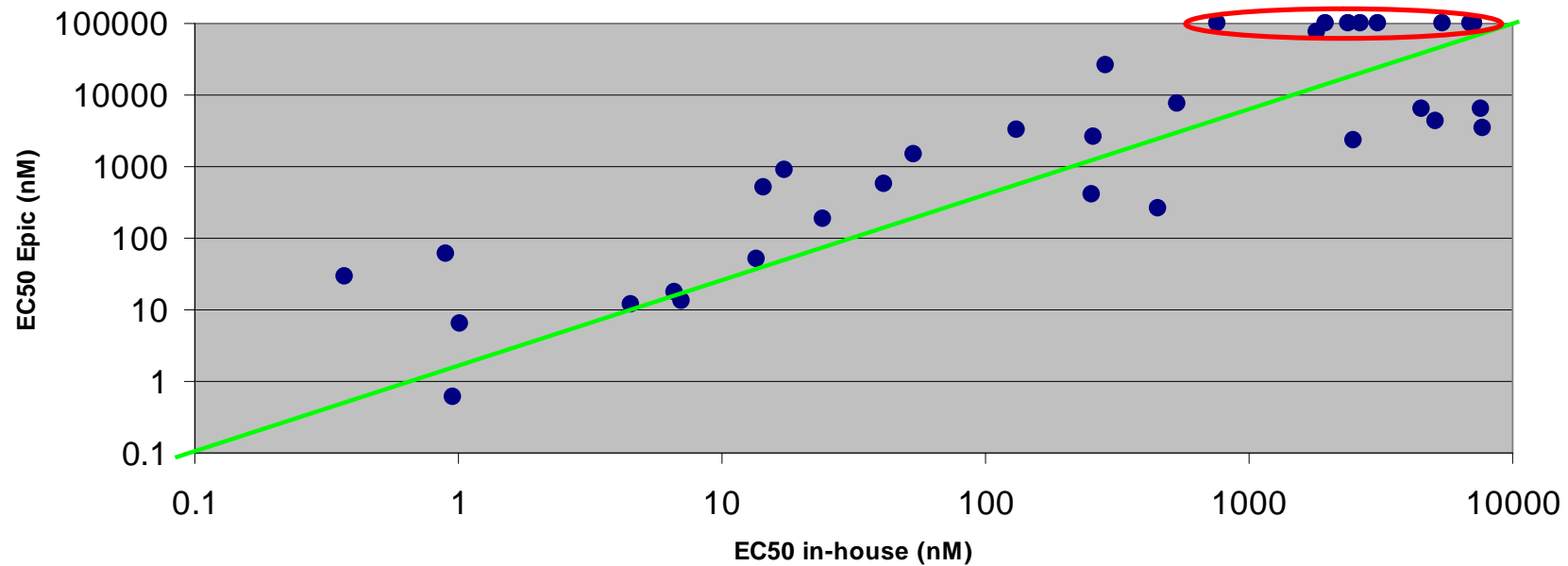
60 minutes: ~ 13 nM

10 minutes: ~ 3 μ M

GPCR 1 (Gs)

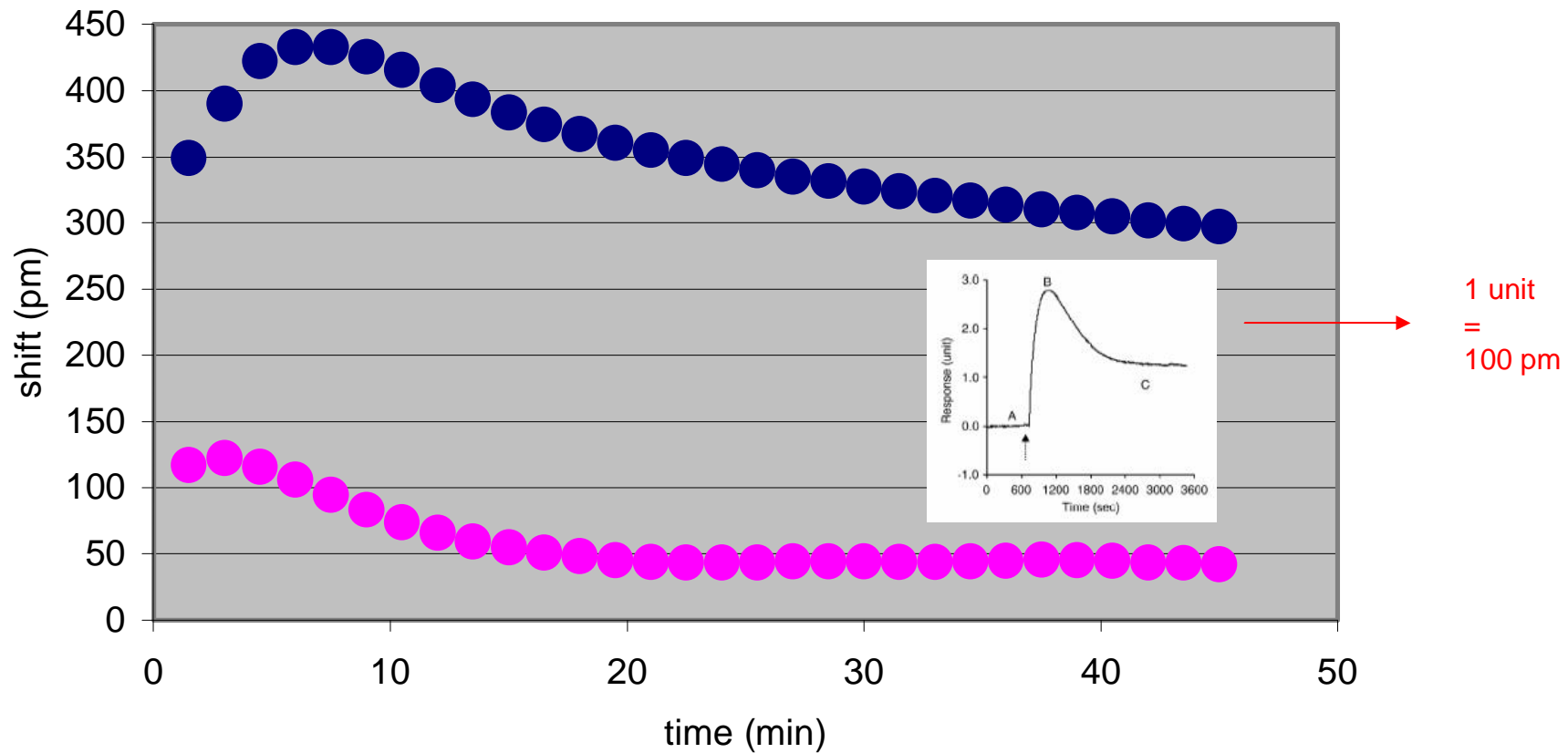
Correlation G_s GPCR with β -lac assay

Note: cmpd solubility issue for some of the cmpds



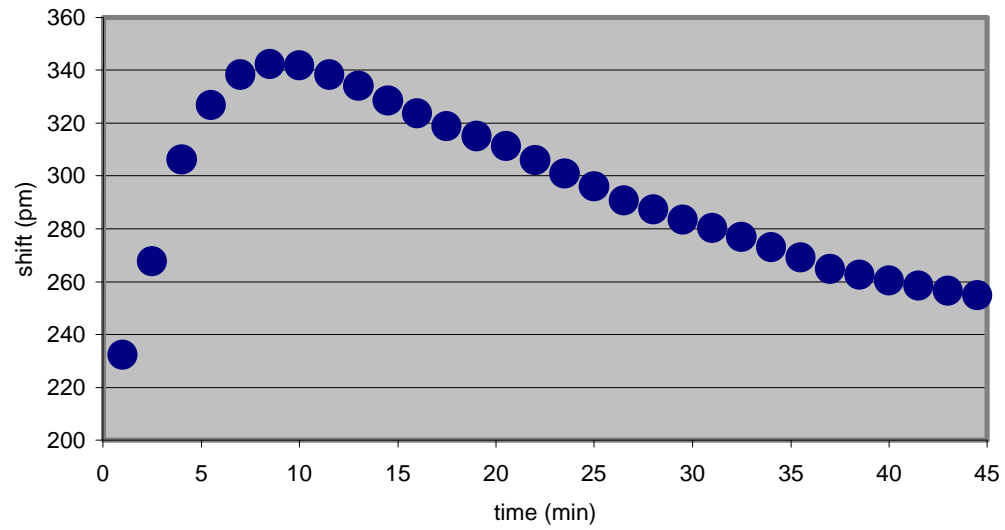
GPCR 1 (Gs)

G_q GPCR signal pattern

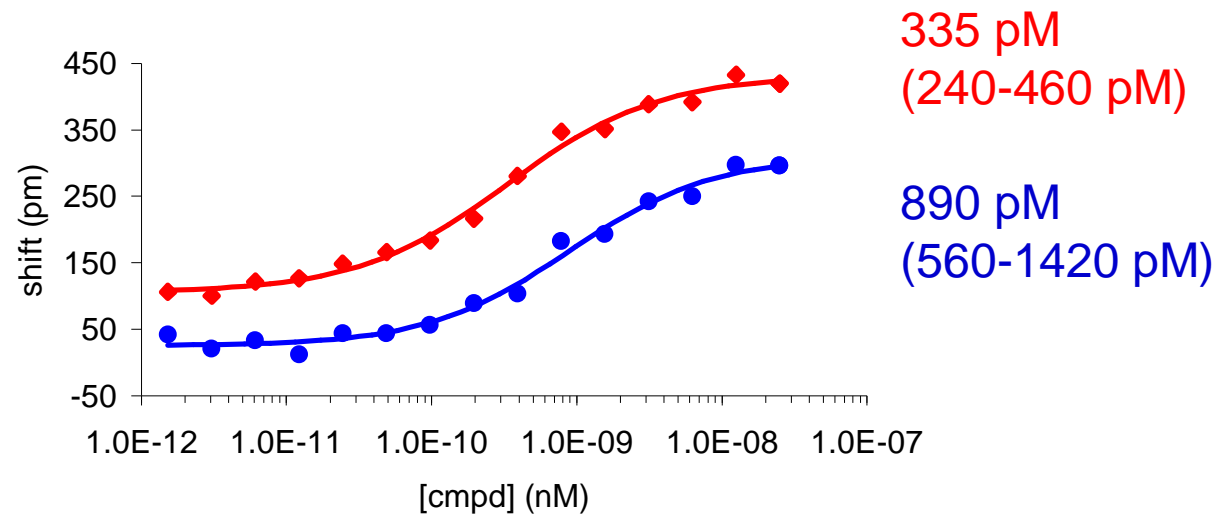


GPCR 2 (Gq)

Assay Window Gq



Standard Cmpd Peak vs End

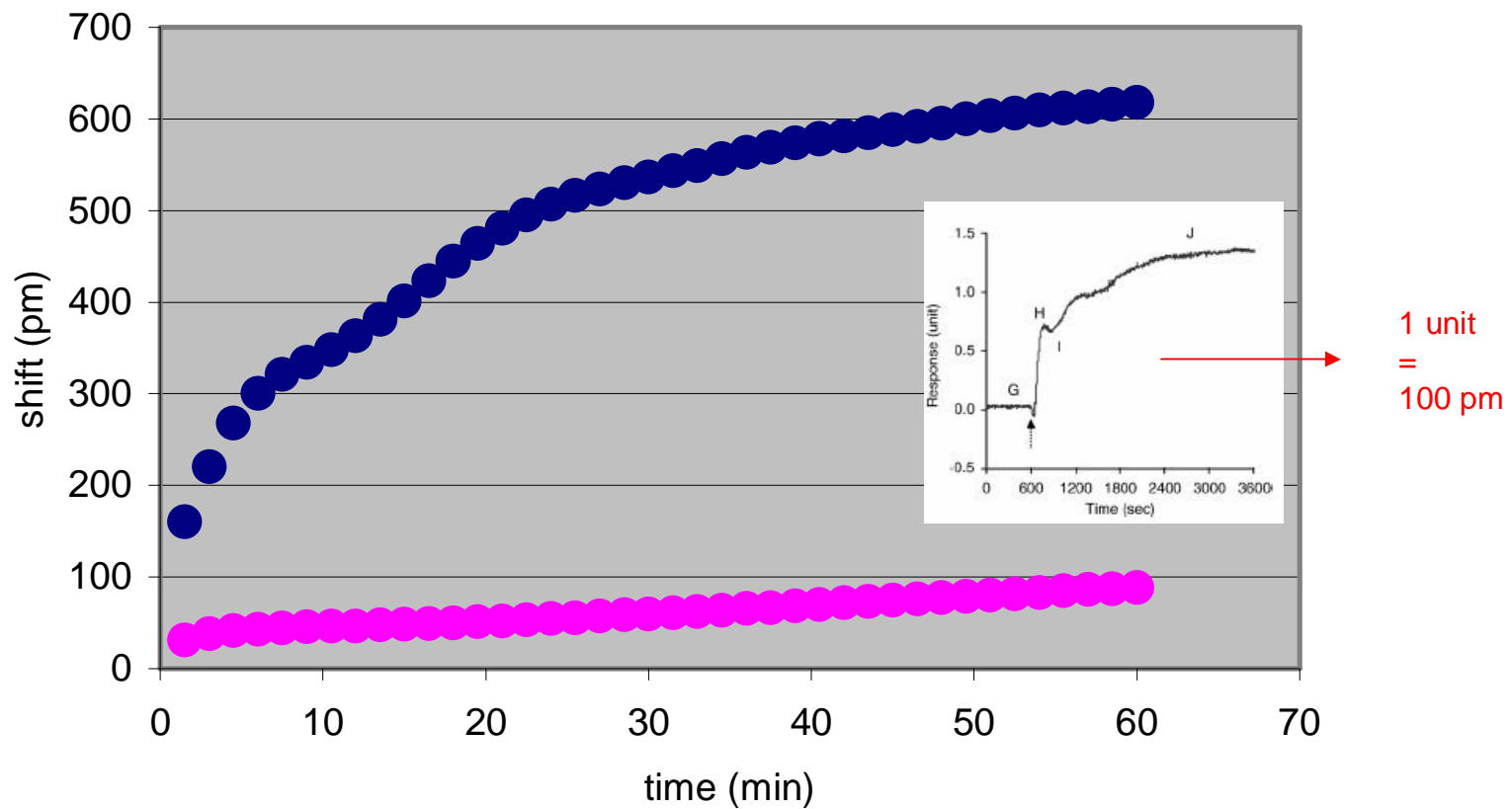


335 pM
(240-460 pM)

890 pM
(560-1420 pM)

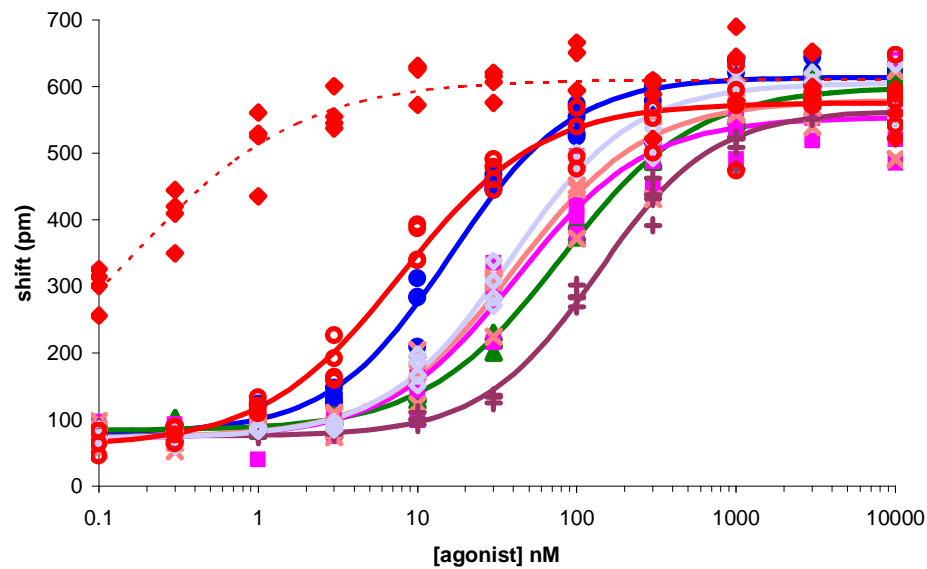
GPCR 2 (Gq)

G_i GPCR signal pattern



Data: Emma Fairman

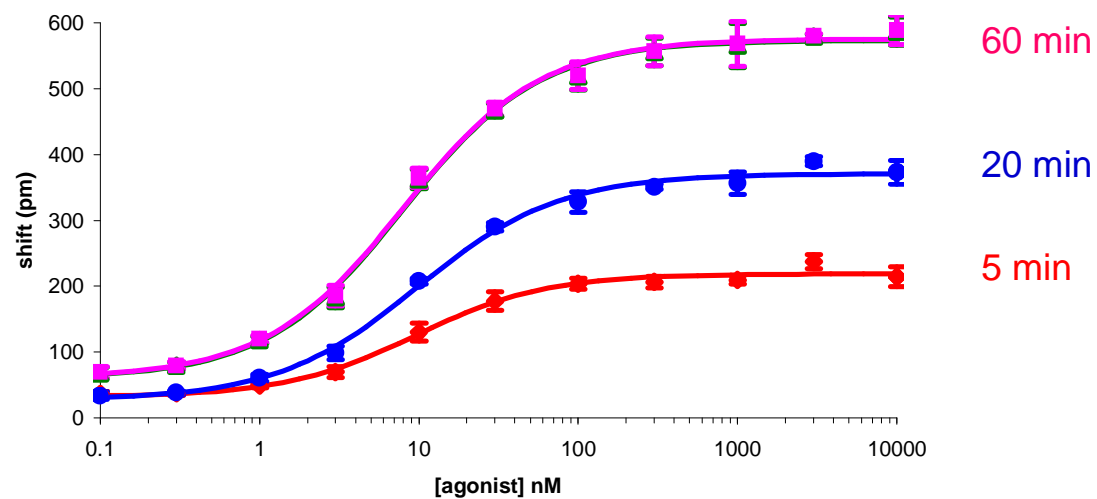
GPCR 3 (Gi)



* no Forskolin needed

Cmpds	Epic	Alpha
1	20	15
2	103	182
3	47	18
4	50	50
5	163	131
6	42	672
7	9	11

EC50 values in nM



Data: Emma Fairman

GPCR 3 (Gi)

Outline

Overview Label Free Technologies

In-House Evaluation(s)

Assessment of Label Free Technologies

Application Label Free Technologies

- Surface Plasmon Resonance (SPR)
 - Low to medium throughput technologies
 - Unique kinetic and thermodynamic data
 - Does not replace biochemical enzyme assays
 - Routinely used to influence enzyme SAR
- Platebased Electrophysiology
 - Low to lower-medium throughput technology
 - Mechanistically correct data for (ligand and voltage gated) ion channels
 - Enabling technology for ion channel targets
 - Routinely used for SAR and focussed screens

- Surface Optical Technologies
 - Robust medium throughput technology
 - High throughput technically possible
 - Non-expert technology
 - Simple assay development process for cell based assays
 - OK for GPCR SAR generation(IC_{50} and single point)
 - Overall good agreement with established pharmacological profile for recombinant systems
 - Complementary readout outside context of classical GPCR theories
 - *Additional high content information on pathway*
 - Most likely superior for Gi coupled GPCRs
 - Can work with endogenous receptors
 - Limited in-house experience with primary cells

“Physiologically Relevant Assays”

Physiologically Relevant Cells

+

Relevant Endpoint

Thorough characterisation

In vivo disease relevance unknown

Choice of endpoint?

Endpoint robust enough for SAR?

Multiple assays for necessary

(Label free) technologies can help to understand assay biology

“Physiologically more relevant assay”

“Physiologically relevant screening cascade”

Any Questions?

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