



# Label free technology: are we there yet?

Accelerating Drug Discovery

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Molecular Devices Tutorial  
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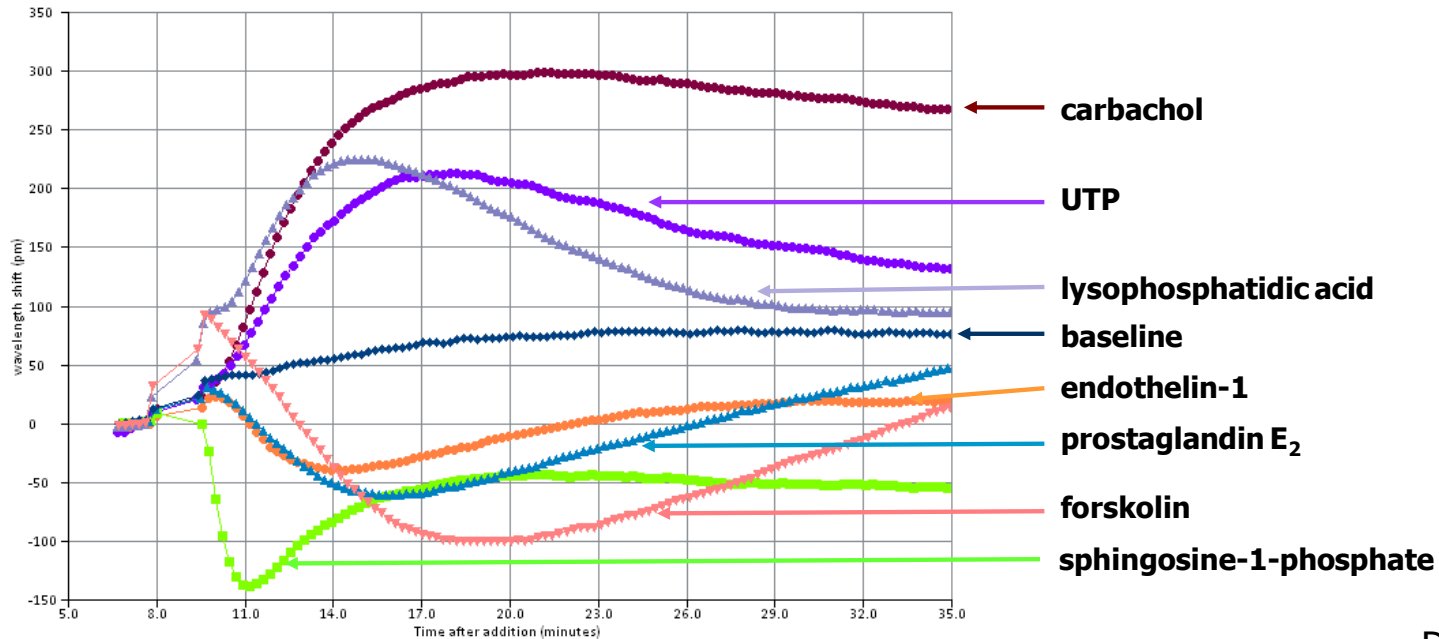




# Overview

- Scope of evaluation – the travel plan
- Additional information obtained – detours ahead
  - changes in endogenous receptor signalling with over-expression
  - influence of growth surfaces
  - resolution of time dependency of signalling pathways
  - resolution of changes in signalling pathway with ligand concentration
- Summary
- Accessing CellKey<sup>384</sup> services at BioFocus

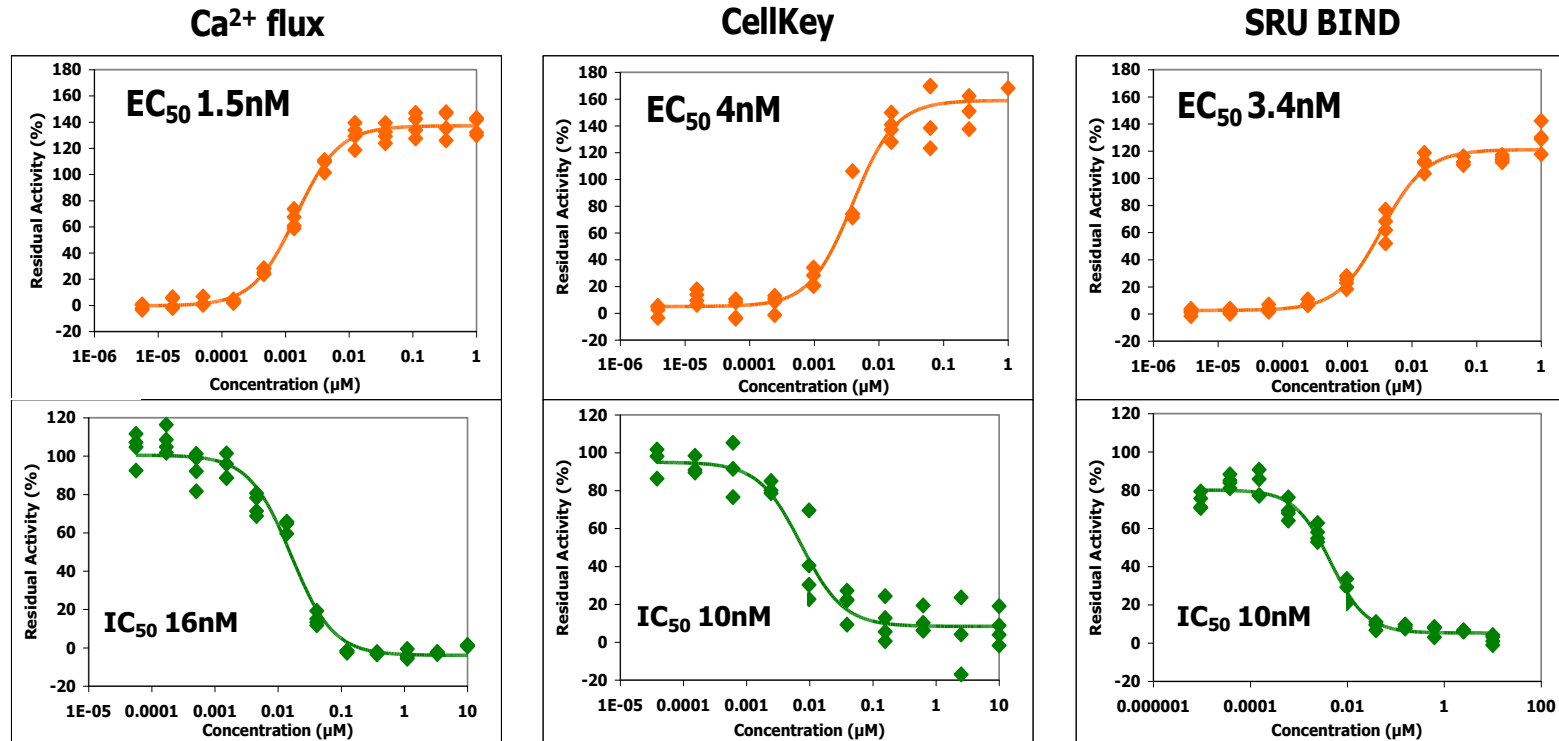
# Delay free start



Data from SRU Bind

- Standard response profiles obtained in HEK cells
  - data confirmed using CellKey<sup>384</sup>
  - robust responses demonstrating expected “fingerprints”

# Robust and reproducible pharmacology



- Over-expressed G $\alpha_q$  coupled receptor in CHO cells
- Data derived very quickly, minimal assay development time

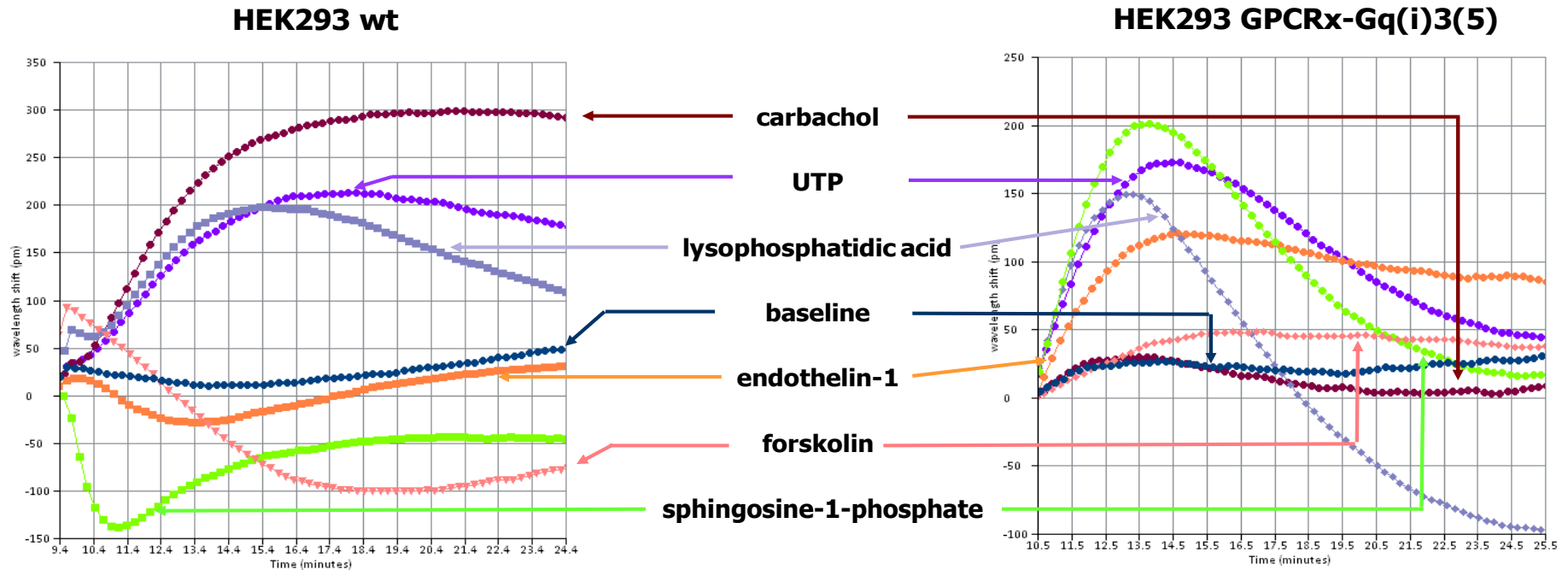


# What can label free add?

## By changing route will we discover new places?

- Very quickly established assay conditions
- Demonstrated consistent pharmacology across platforms
- What additional data could be derived
  - what effect does receptor over-expression have?
  - can we unravel signalling routes?
  - is an assay plate just an assay plate?
- What value does this add to the drug discovery process?

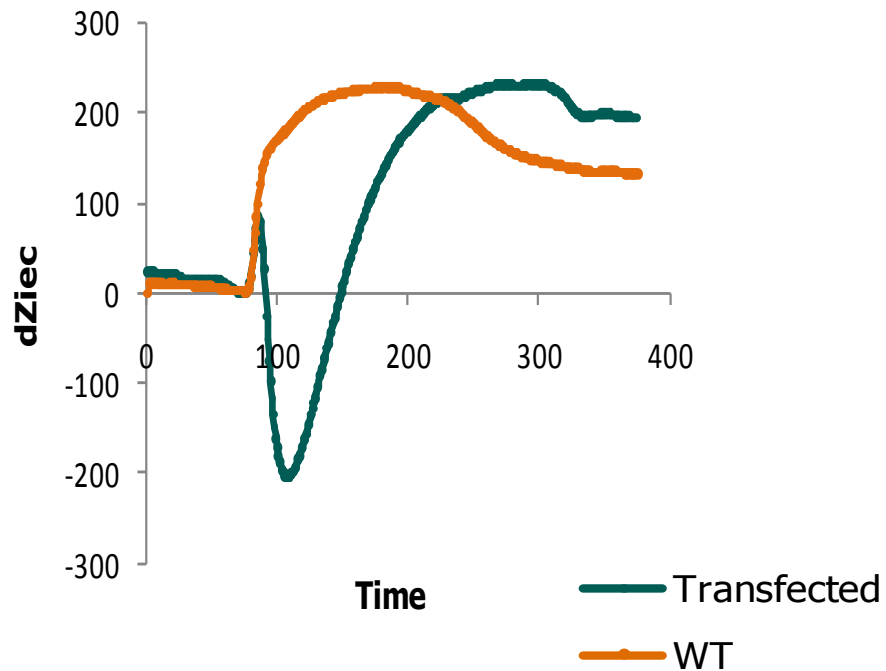
# Endogenous signalling pathways



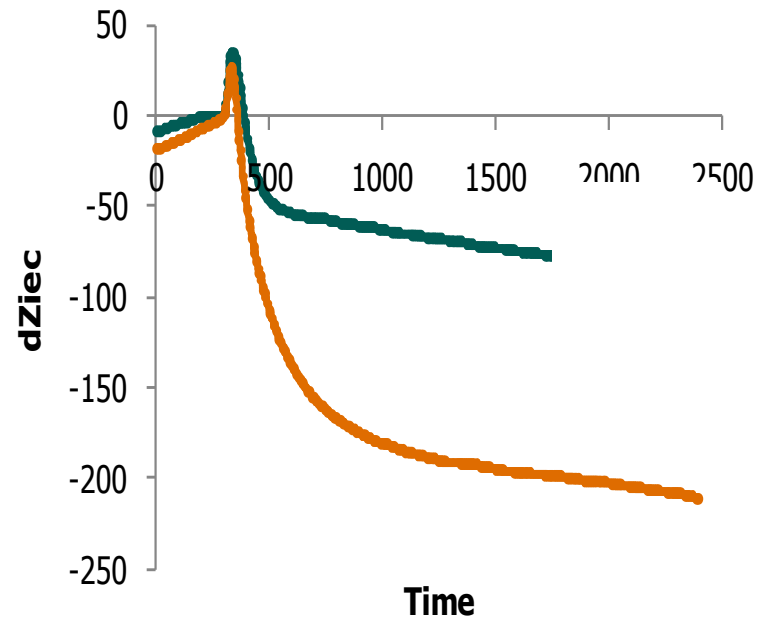
- Response profile is altered independently of anticipated coupling mechanism
- Is this related to platform or over-expression of G-protein or receptor?

# Over-expression alters endogenous signalling

Carbachol responses in transfected and non-transfected HEK cells

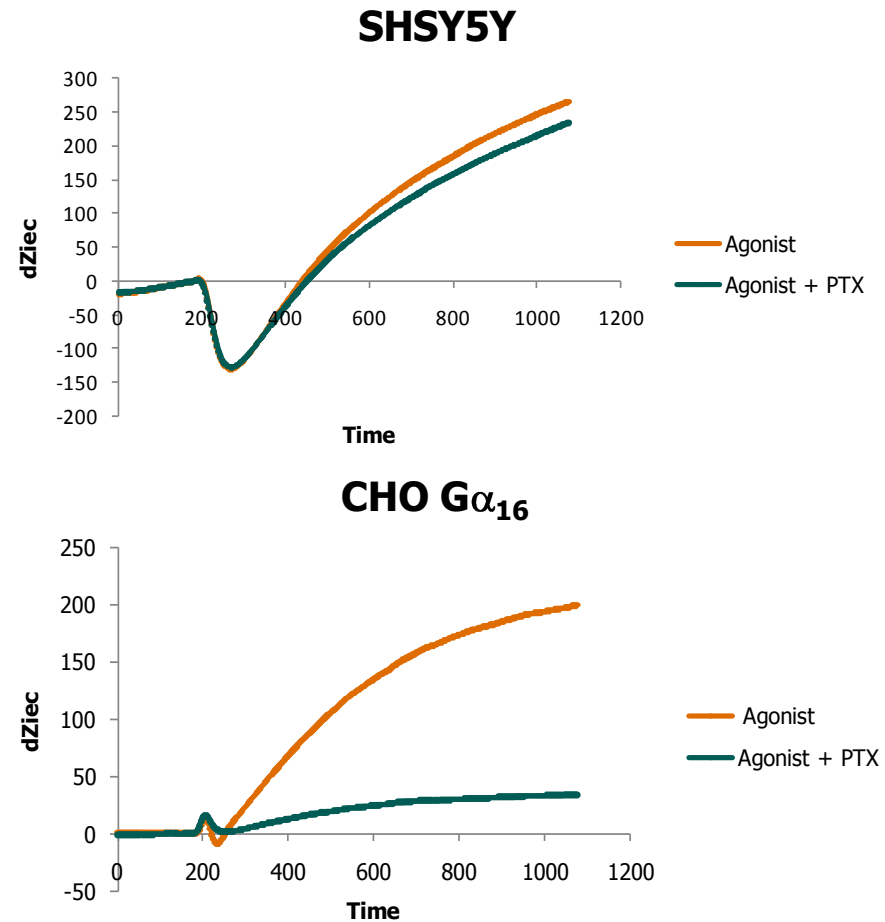


PGE2 responses in transfected and non-transfected CHO cells

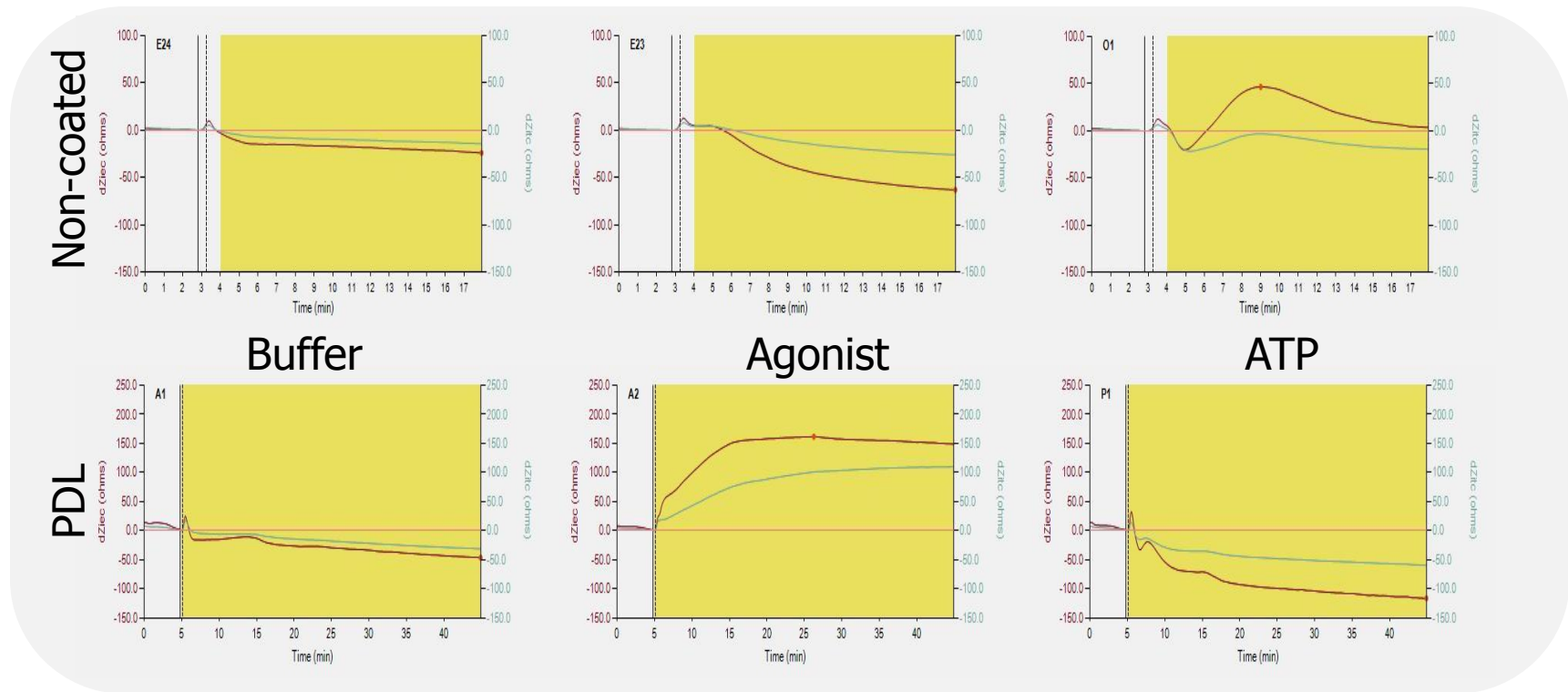


# Cell background influences response

- Same receptor shown to couple to multiple signalling pathways
  - 1  $\mu\text{M}$  ligand used
- Scale of response flattened in CHO  $G\alpha_{16}$  cells
- Pertussis toxin markedly modulates response in CHO  $G\alpha_{16}$  but not SH-SY5Y neuroblastoma cells
- Cell background influences response fingerprint

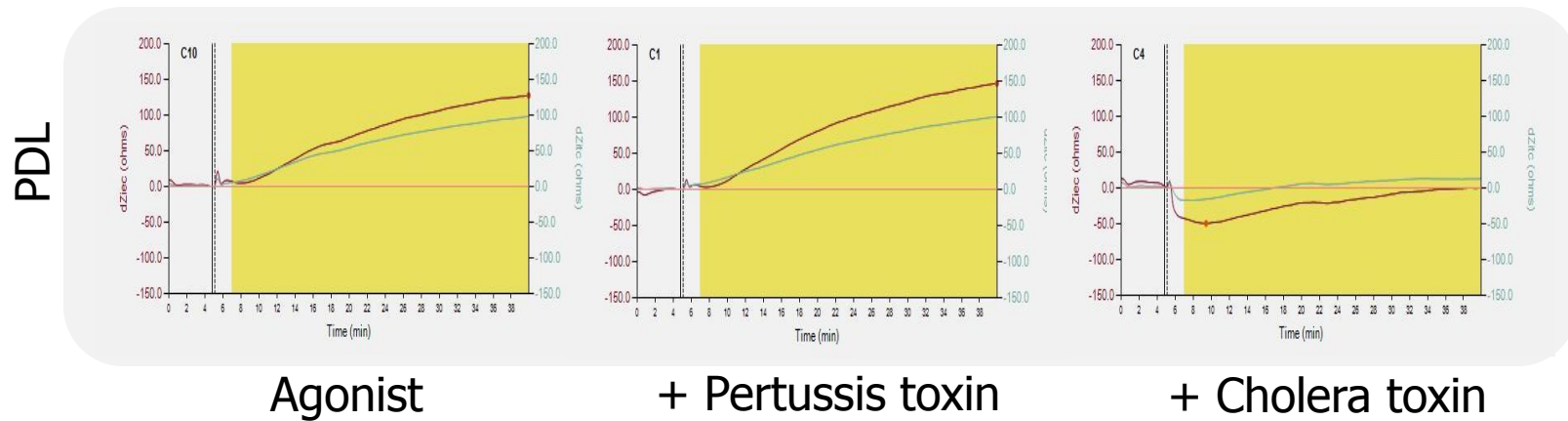


# Not all growth surfaces are equal



- Change in coating can dramatically affect impedance response
  - transfected and endogenous responses both changed
  - is this alerting us to a change in coupling mechanism?

# Modulation: the key to understanding

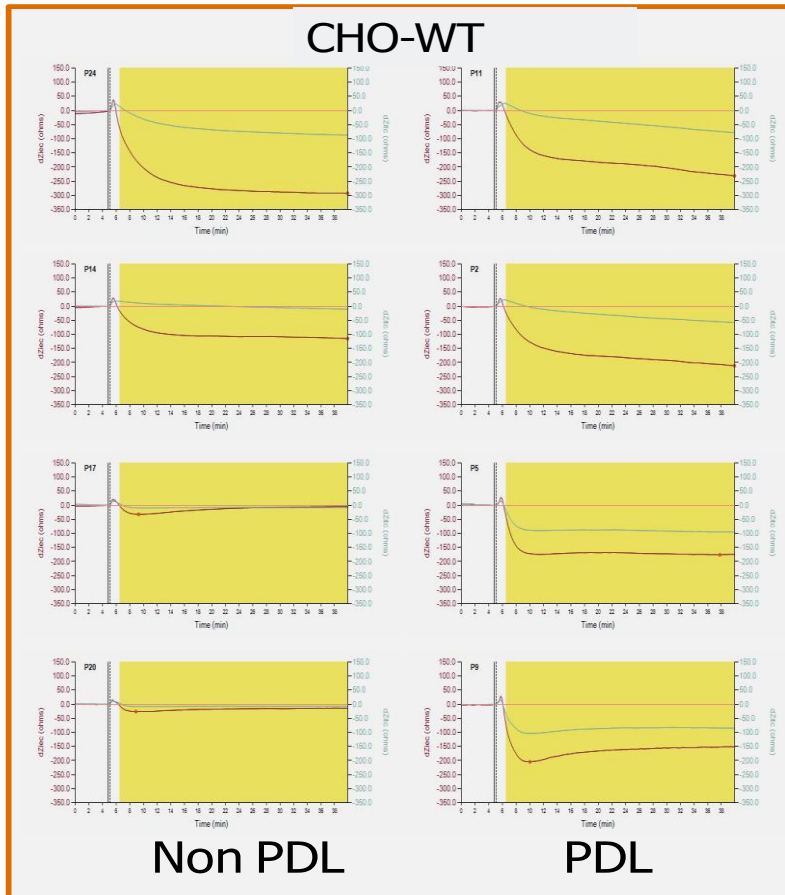


- Directional change in dZiec does not necessarily mean a change in signalling
  - modulation by cholera toxin implies GPCR still coupled through  $G\alpha_s$
- What happens with endogenous  $G\alpha_s$  response?
  - is over-expression of receptor contributing to the “flip” in response?



# Plate coating and endogenous responses

## PDL affects ability to modulate PGE2 response



PGE2  
only

+PTX

+cholera

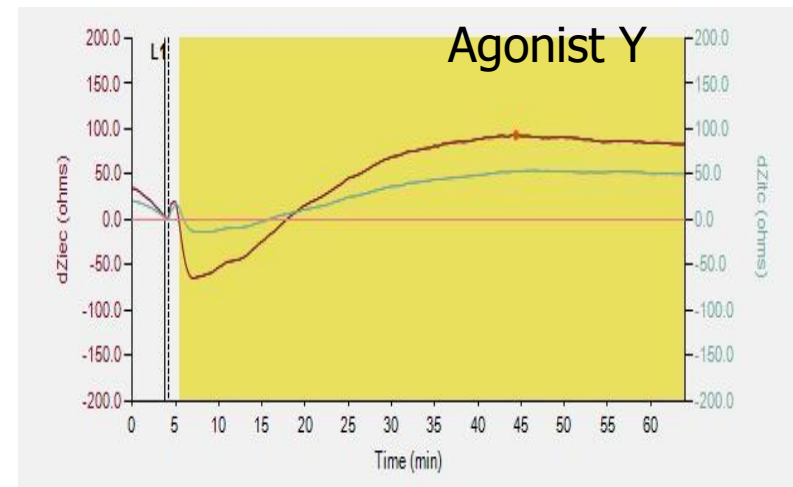
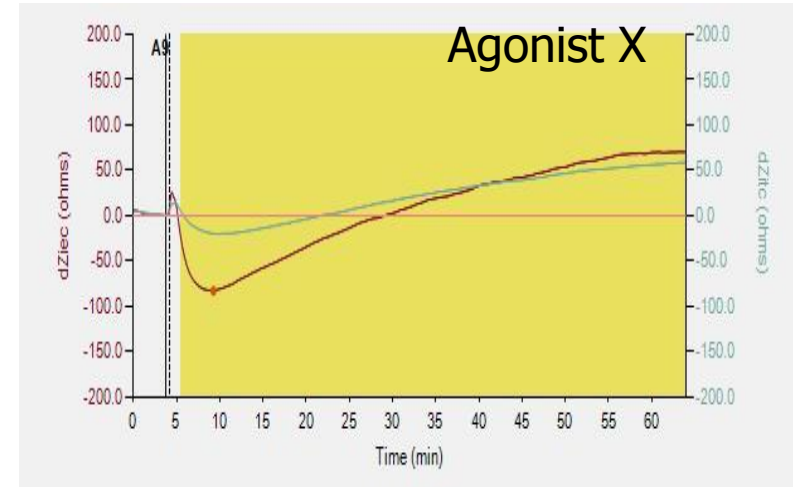
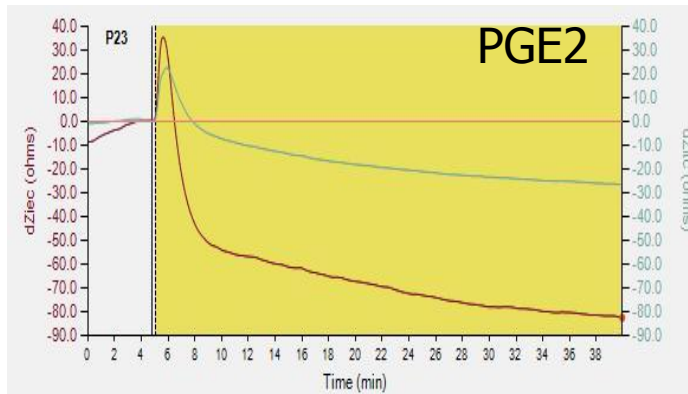
+PTX  
+cholera

- Impedance profile not reversed with PGE2 stimulation
  - contrasts with the response for an over-expressed receptor
- Cholera toxin modulation of the PGE2 response only in the absence of poly-D-lysine

# Differential signalling with time

## Increasing read time reveals opposing signal events

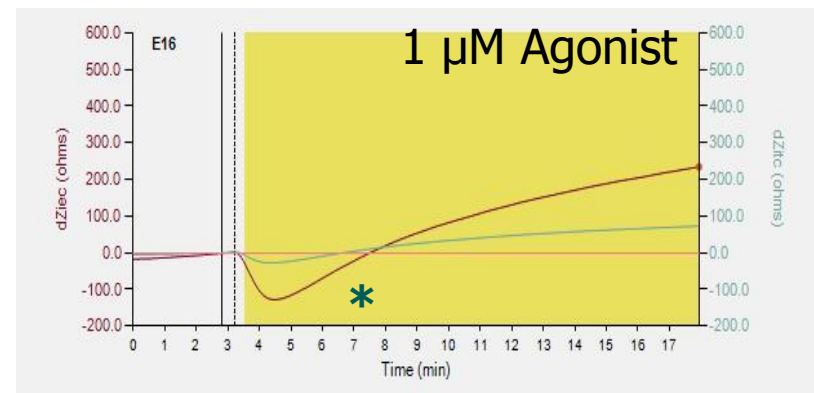
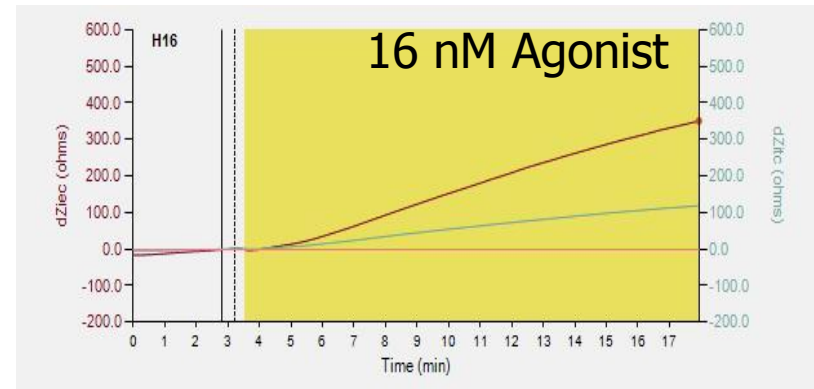
- CHO cells over-expressing a  $G\alpha_s$  coupled receptor
- Transition from  $G\alpha_s$  to  $G\alpha_i$  after 30 minutes of stimulation
  - not seen with endogenous receptor (PGE2)
- Would not be detected using cAMP accumulation assay



# Differential signalling in SH-SY5Y cells

## Change in pathway with increasing ligand concentration

- Peptide receptor over-expressed in neuroblastoma line
  - reported to couple  $G\alpha_i$  and  $G\alpha_q$
- Shift between pathways is dependent on concentration of ligand
- Attempts to modulate with BAPTA, U73122 and pertussis toxin
  - further work required





# Fascinating stuff, but.....

## Possible implications for drug discovery?

- Careful choice of cells for controls
  - non- or mock transfected cells may not be an appropriate control
- Presence of factors affecting signalling pathway may mislead compared to native situation
- Revelation of signalling events overlooked by conventional methods
  - opposing or time dependent effects
  - surface induced effects on cell behaviour



# Has the journey been worthwhile?

## Strengths

- Reveals complexity of signalling
- Study of multiple signalling events in one well
- Kinetic information
- Allows for modulation of specific parts of pathways
- Identification of differences between diseased vs normal cells

## Challenges

- Reveals complexity of signalling
- Lack of specific modulators of signalling cascades
- Time to analyse data
- Complexity of data analysis
- Clear experimental aim



# Are we there yet?

- Data sets are indicative of higher level of information available
  - identifies events over looked by “conventional” methods
  - findings consistent between platforms and other groups
- Ability to interpret signalling is limited by available tools
  - the questions are easy, it’s the answers that are tricky
- Additional information provided is valuable
  - highlights weaknesses in existing approaches
- Data requires careful interpretation
  - careful experimental design
- CellKey<sup>384</sup> available on fee-for-service basis



# Acknowledgements

## Chesterford Park:

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Jayne Ingram

Ryan McGuinness



# Additional information

## BioFocus booth #835

Drug discovery service capabilities, including CellKey<sup>384</sup>

## Poster #C324

Label free technology: value added information in the drug discovery process

## Poster#C323

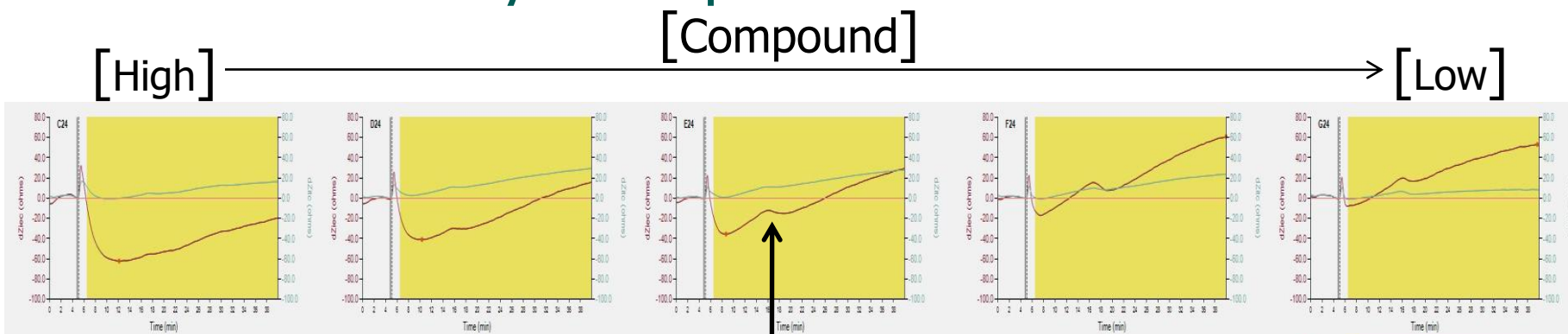
Label free technology: the weird and wonderful world of cell signalling



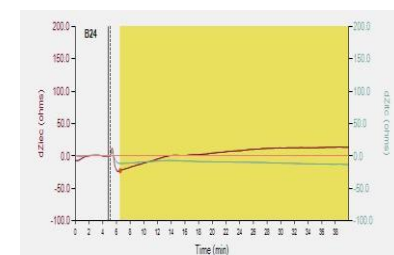
# APPENDIX

# Validation of hit compounds

## Careful data analysis required



Inflection point coincides  
with  $G\alpha_s$  to  $G\alpha_i$  "switch"



buffer

- CHO cells over-expressing a  $G\alpha_s$  coupled receptor
- Subtleties in the "fingerprint" of active compounds likely reveal important mechanism of action information
- Can a single analysis metric be applied?
  - what time point and what metric?