

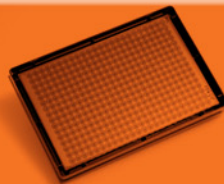
CORNING

Epic[™]
system



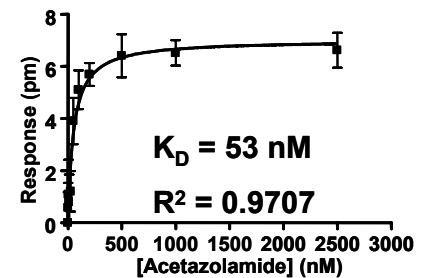
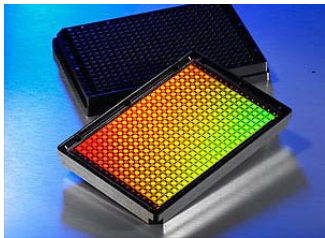
Outline

- Epic™ System Overview
- HTS Operation & Integration
- Biochemical Assays
- Cell-Based Assays
- Summary



Corning[®] Epic[™] System

- A label-free drug screening system
 - A 384-well microplate with optical biosensors & surface chemistry
 - An HTS-compatible microplate reader



Epic[™] Microplate

- 384-well format
- Optical biosensor in each well
- Surface chemistry

Epic[™] Plate Reader

- Compatible w/ HTS automation
- $\geq 40,000$ wells/8hrs
- Sensitivity of $5\text{pg}/\text{mm}^2$ (assay dependent)

Binding Data

- Manipulated and analyzed by customer

Epic™ System Key Attributes

■ Label-Free Detection

- No interference due to presence of a label or dyes
- A priori info about natural ligand not required

■ High-Throughput

- 40,000 wells in 8 hours
- 384-well microplate format
- Easily integrated with existing automation/instrumentation

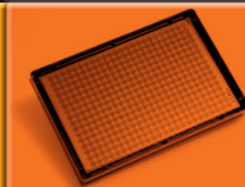
■ Broad Capability

- Biochemical assays (Yes/No Binding; affinity ranking; direct bind and functional)
- Cell-based assays

■ High Sensitivity

- Small molecule (157Da) binding to protein targets
- Cellular response for *endogenous* receptors, including primary cells

INSTRUMENT FUNCTIONALITY

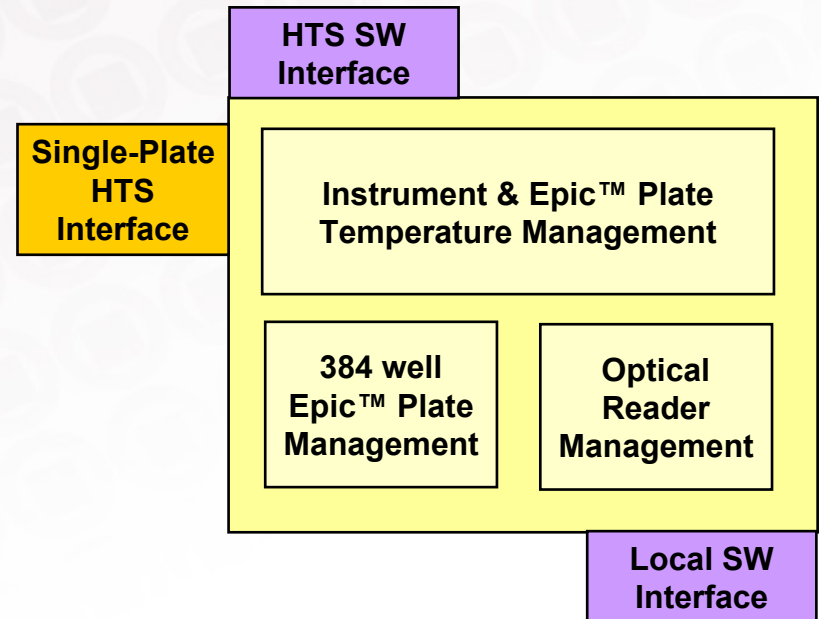


Commercial Instrument Functionality

Commercial Instrument

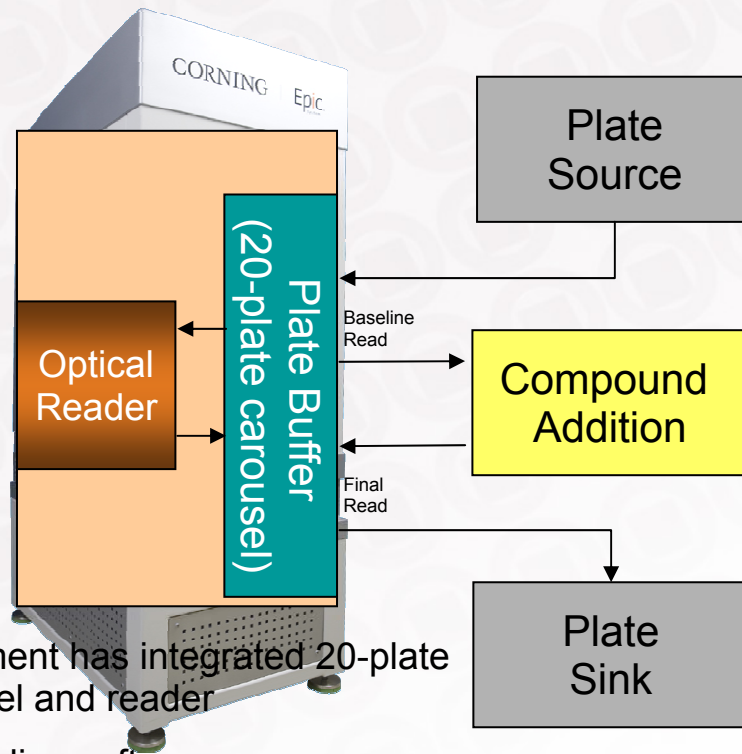


- Reader only
- Internal temp. control



Epic™ System HTS Operation

Epic™ Instrument



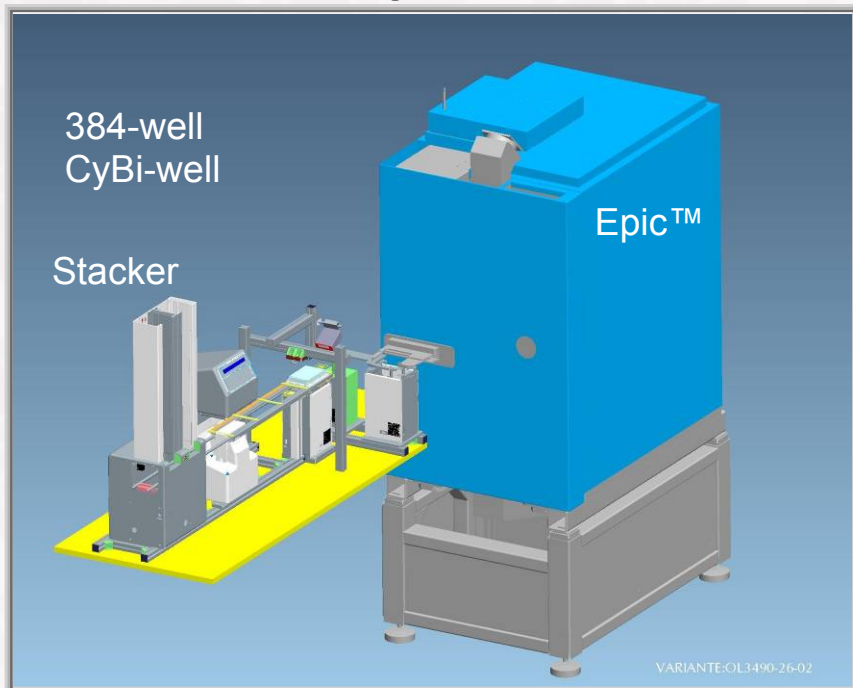
- Total Epic™ cycle time is approximately 4.6min/plate, enabling up to 40,000 wells in 8 hours

Process Step	Time (min)
Load	0.2
Baseline Read	1.9
Unload	0.2
Compound Addition	-
Reload	0.2
Final Read	1.9
Unload	0.2
Total (min/plate)	4.6

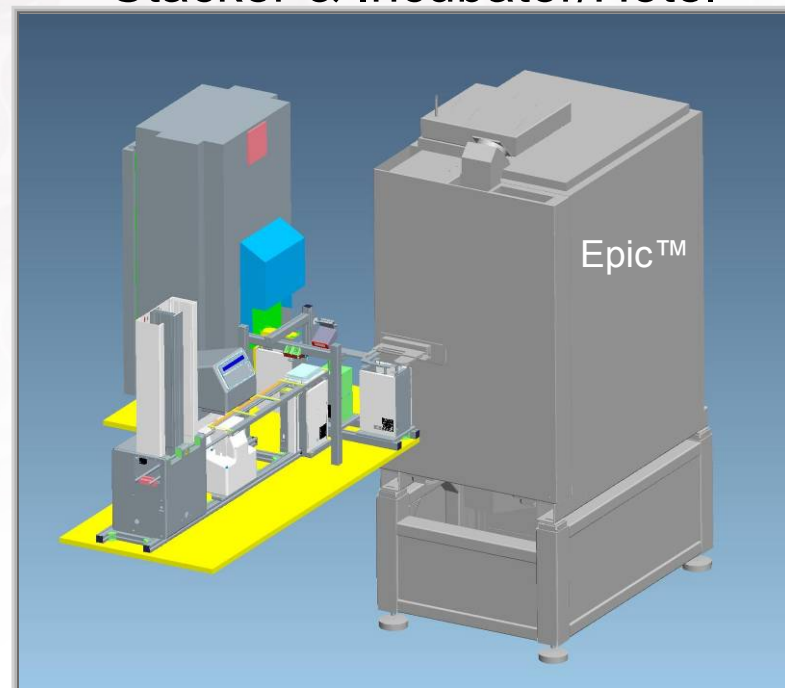
- Instrument has integrated 20-plate carousel and reader
- Scheduling software accesses reader and carousel independently to maximize throughput

Epic™ System Can Easily be Integrated with Other Instrumentation

Integrated Liquid Handling & Stacker



Integrated Liquid Handling, Stacker & Incubator/Hotel



Applications of the Epic™ System

Biochemical Assays

- Protein/Drug
 - kinase/drug
 - protease/drug
 - carbonic anhydrase/drug
 - human serum albumin/drug
- Protein/Protein
 - cytokine/cytokine receptor
 - antigen/antibody
- Peptide/Drug
 - ala-ala/vancomycin

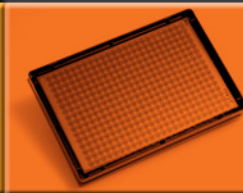
Cell-Based Assays

- Signal Transduction
 - epidermal growth factor receptors
 - G protein coupled receptors
 - cytoskeleton modulations
- Toxicity Screening
- Lipid Signaling
- Proliferation

Hybrid Assays

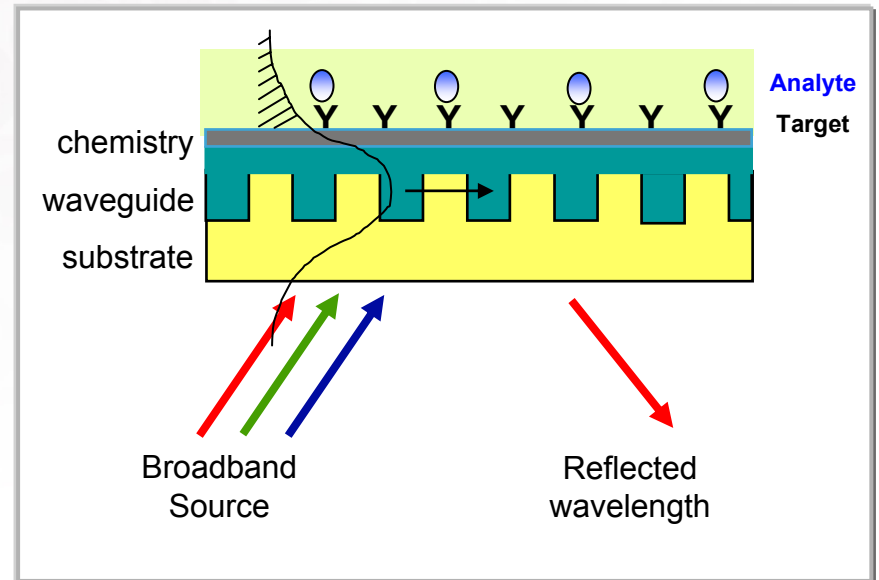
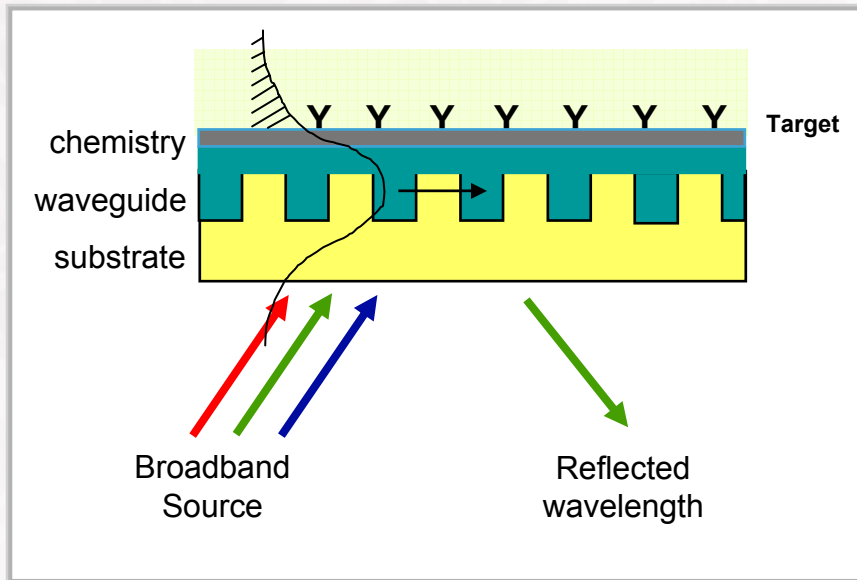
- Whole Cell Lysate
 - Cytokine detection

BIOCHEMICAL ASSAYS



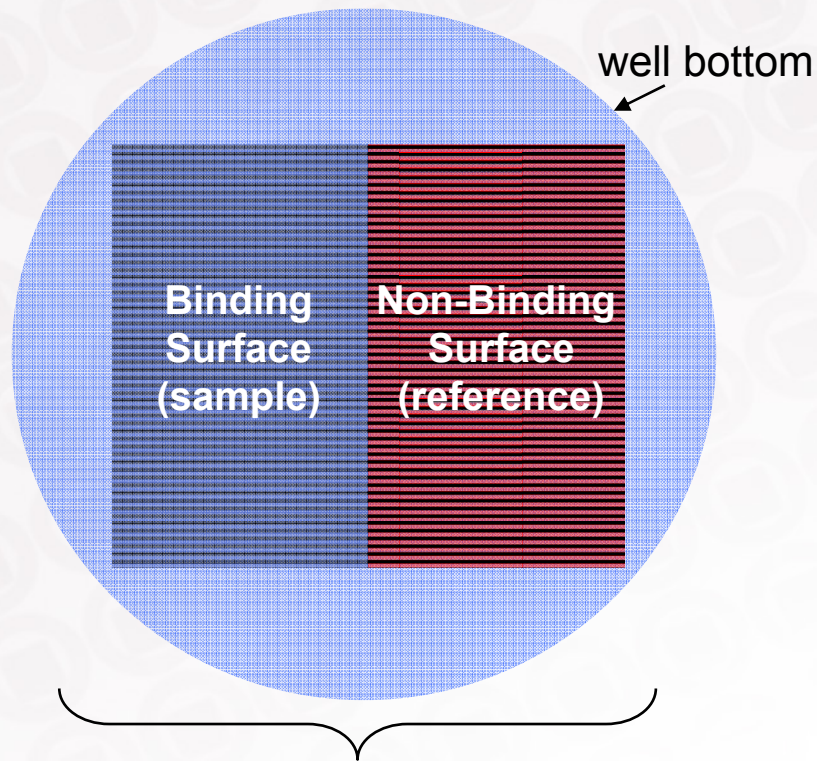
Operating Principle: Biochemical Assays

- Measures changes in index of refraction upon a binding event
- Change in index manifest by a shift in resonant wavelength
- Sensitive to the first ~150nm from the sensor surface

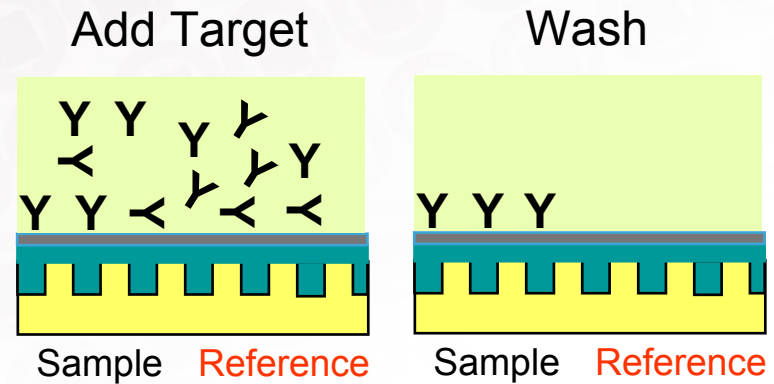


Patented Self-Referencing Technology

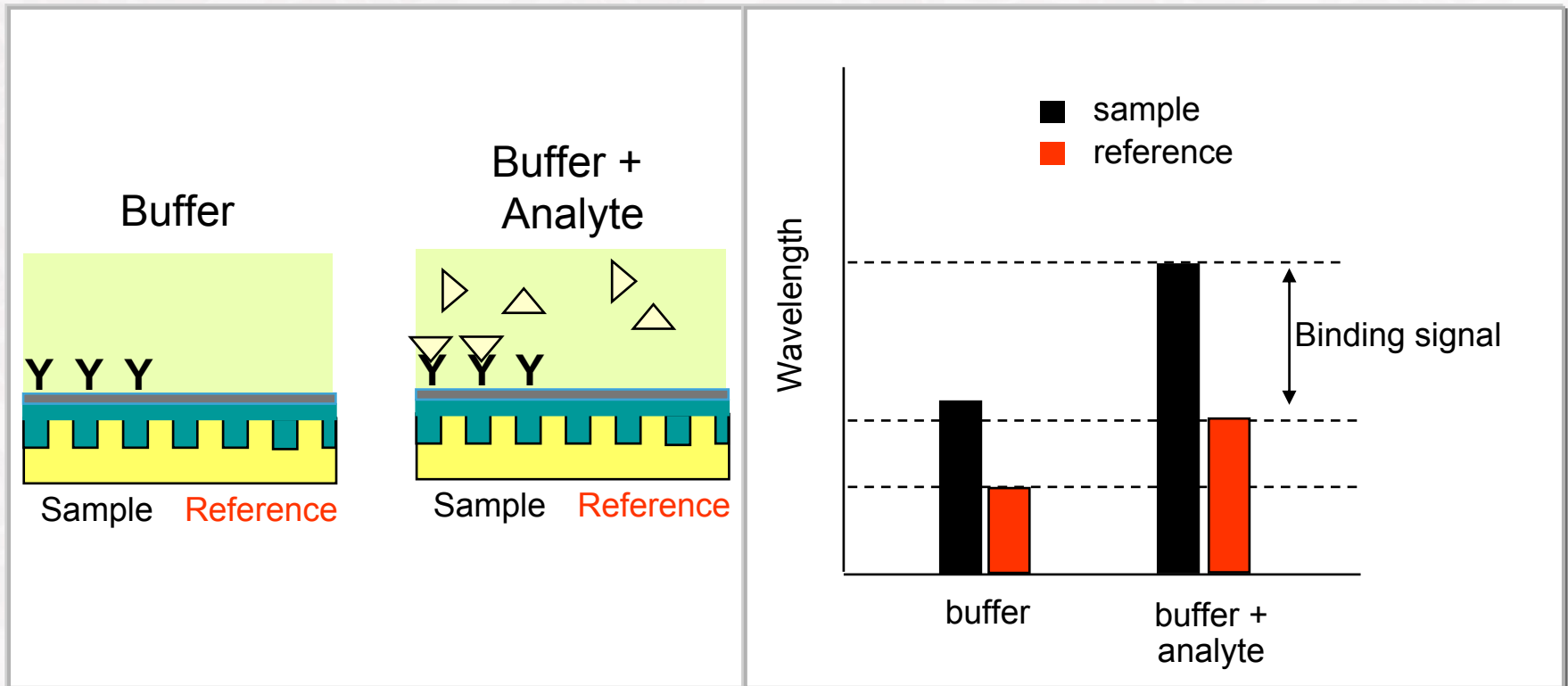
- Utilizing a split sensor (binding/non-binding) referencing scheme:



Single split sensor within well



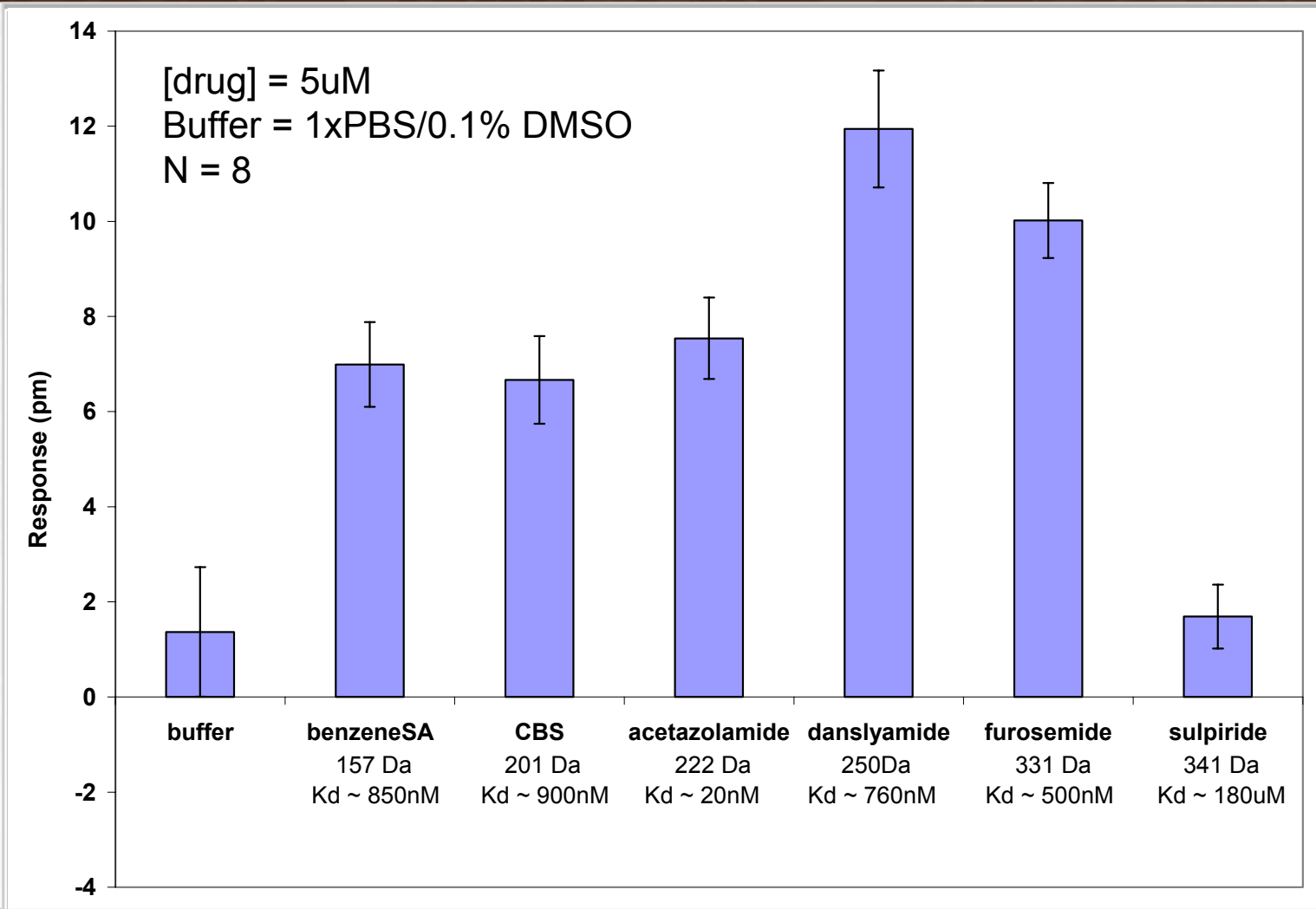
Epic™ System Biochemical Assays Data Analysis



$$\text{Binding Signal} = \text{Sample} - \text{Reference}$$

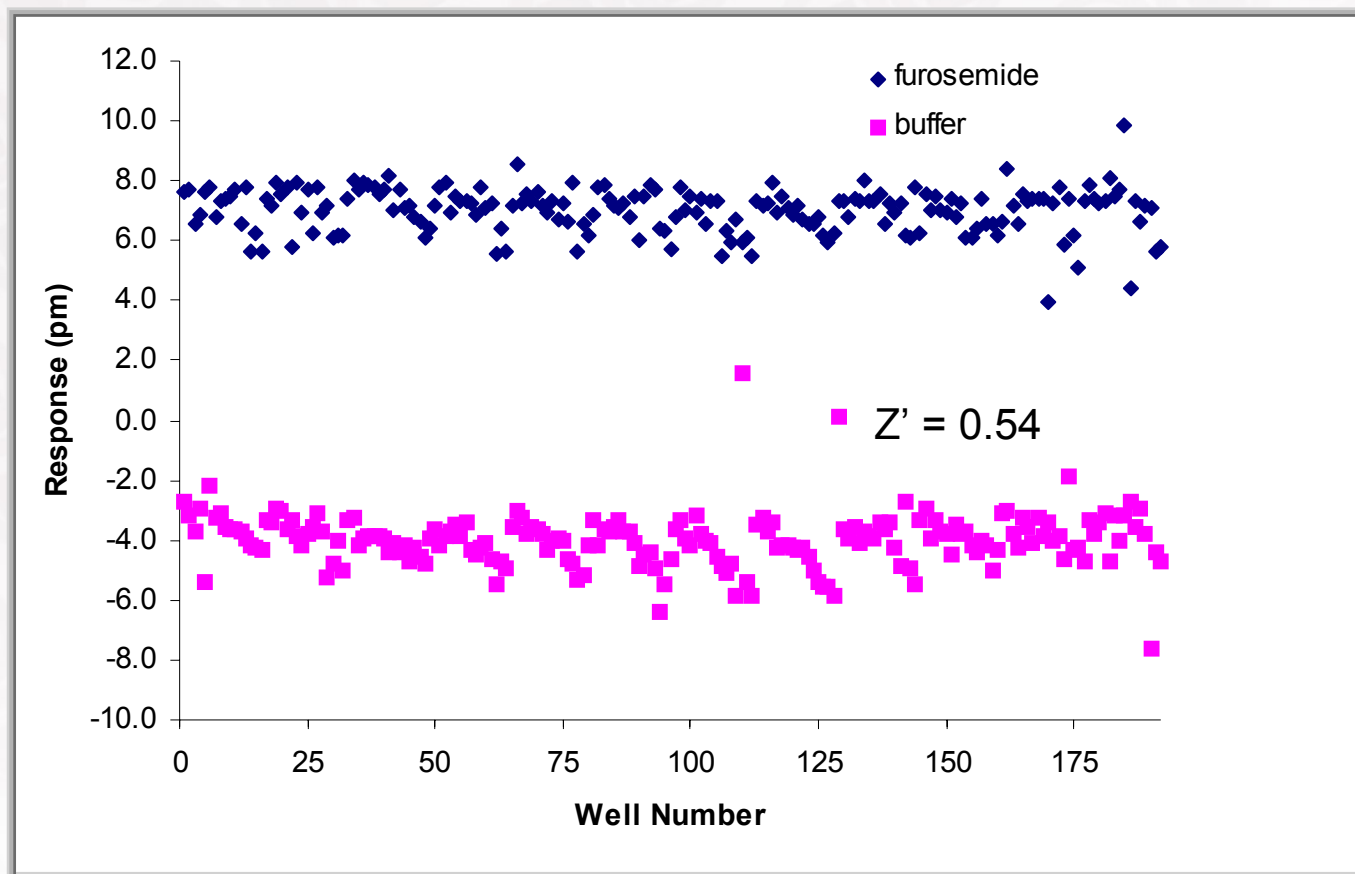
Drug Binding to Carbonic Anhydrase

Yes/No Binding



HTS Small Molecule Assay on Epic™ System: *Furosemide (331 Da) Binding to Carbonic Anhydrase*

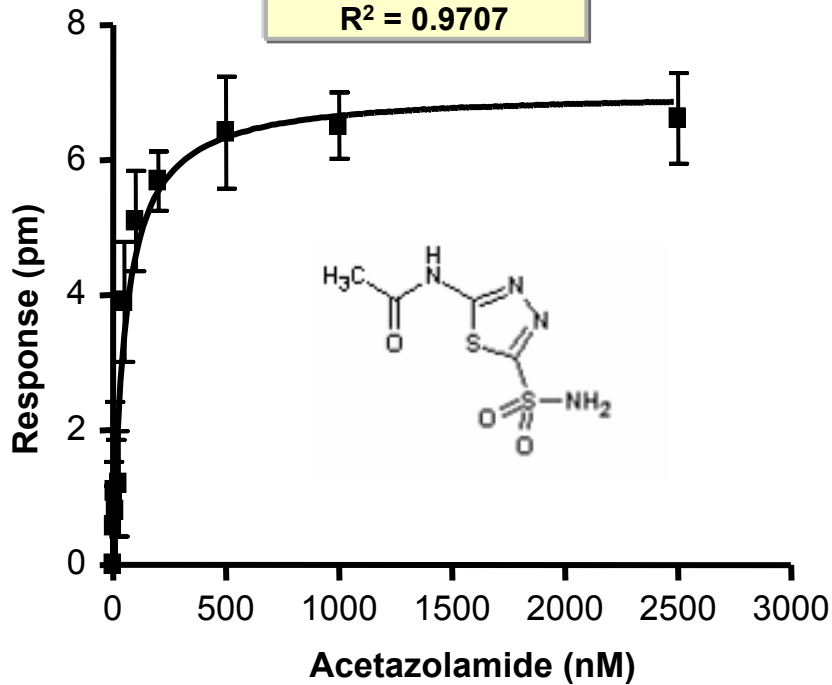
- Good assay performance demonstrated with furosemide binding to carbonic anhydrase in high throughput (0.1% DMSO)



Carbonic Anhydrase Assay K_D Determination

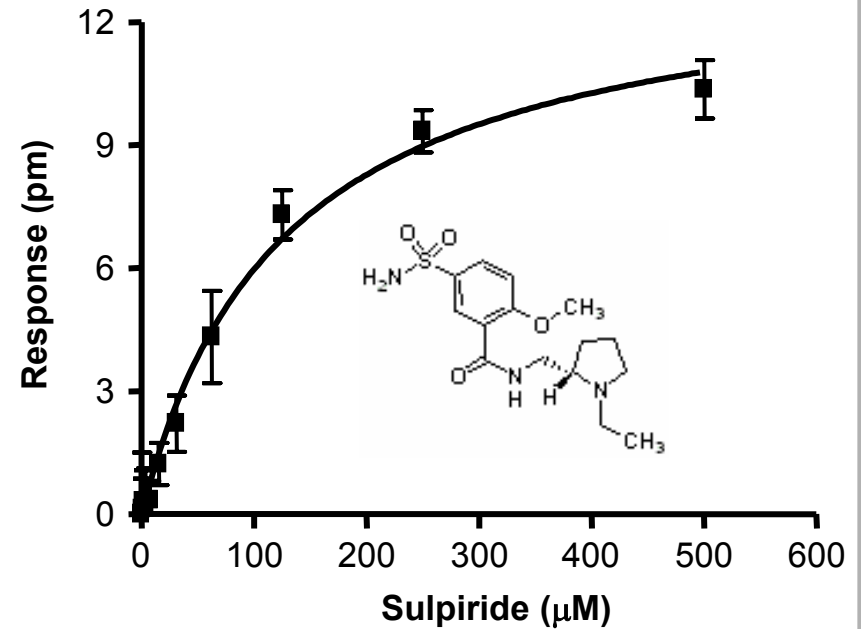
Acetazolamide (222 Da)
Literature K_D : 19nM

$K_D = 53 \text{ nM}$
 $R^2 = 0.9707$



Sulpiride (341 Da)
Literature KD : 186 μM

$KD = 127 \text{ }\mu\text{M}$
 $R2 = 0.9922$



Carbonic Anhydrase Assay

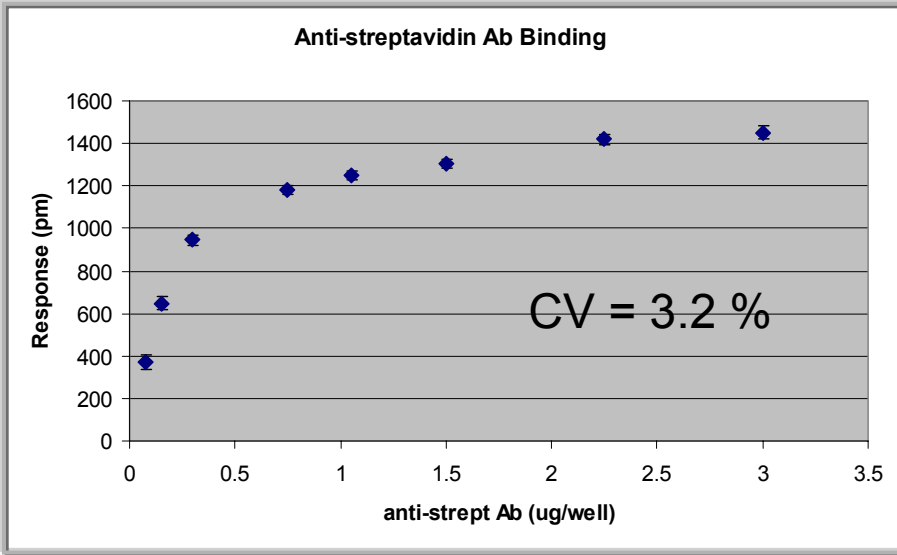
Accuracy of K_D Estimation

Compound	Lit K_D **	Epic™ K_D
acetazolamide	19nM	53nM
furosemide	513nM	534nM
dansylamide	760nM	795nM
sulpiride	186uM	127uM

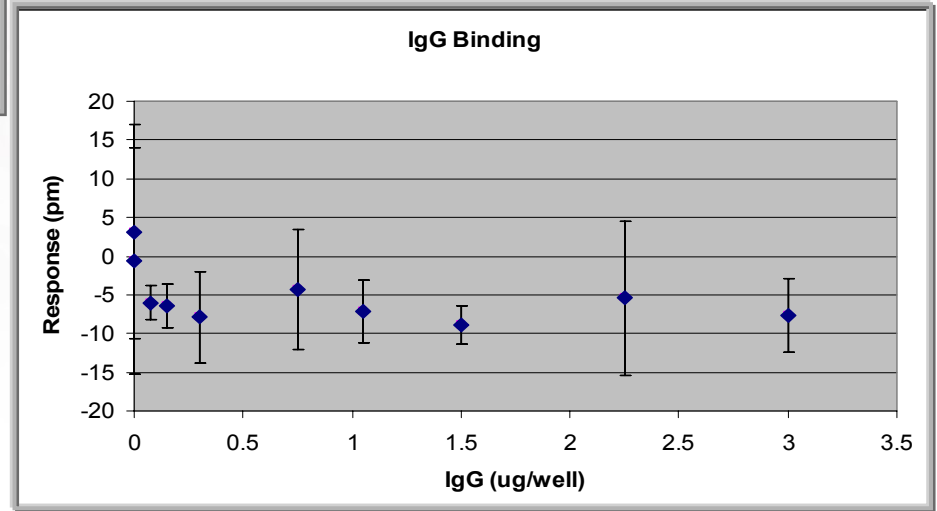
** Literature values are based on Biacore SPR measurements
(see “Analysis of small-molecule interactions using Biacore S51 technology”,
Anal. Biochem 329, 316-323 (2004))

Antibody/Antigen Assay

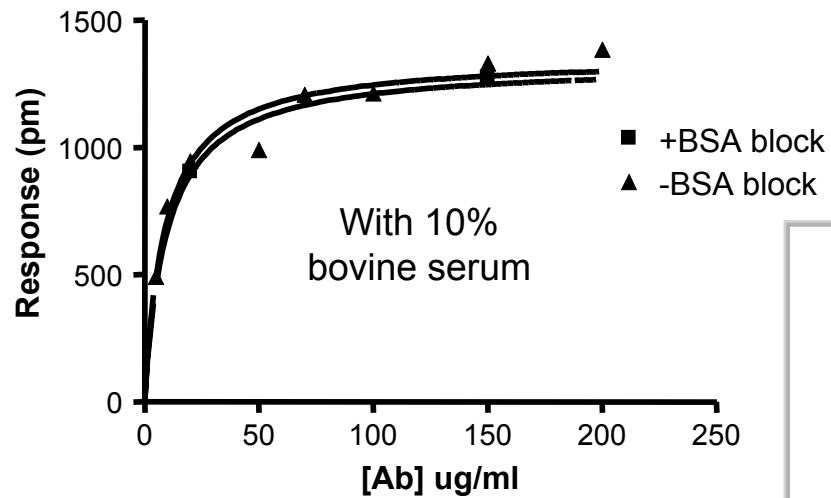
Binding of Anti-Streptavidin Ab to Streptavidin



No IgG Binding

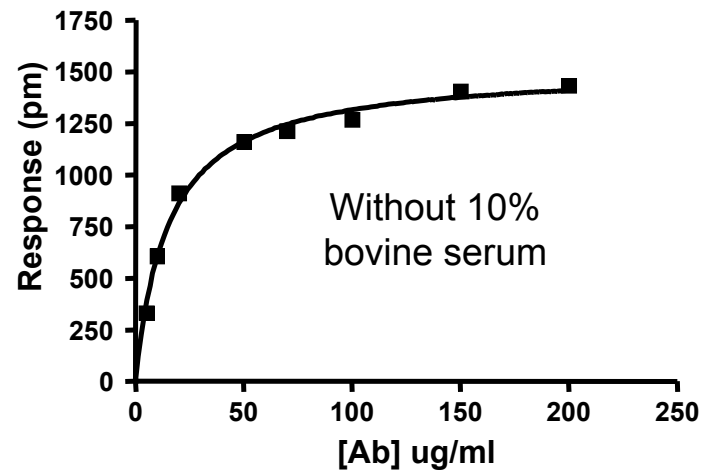


Estimation of Antibody Affinity Complex Samples



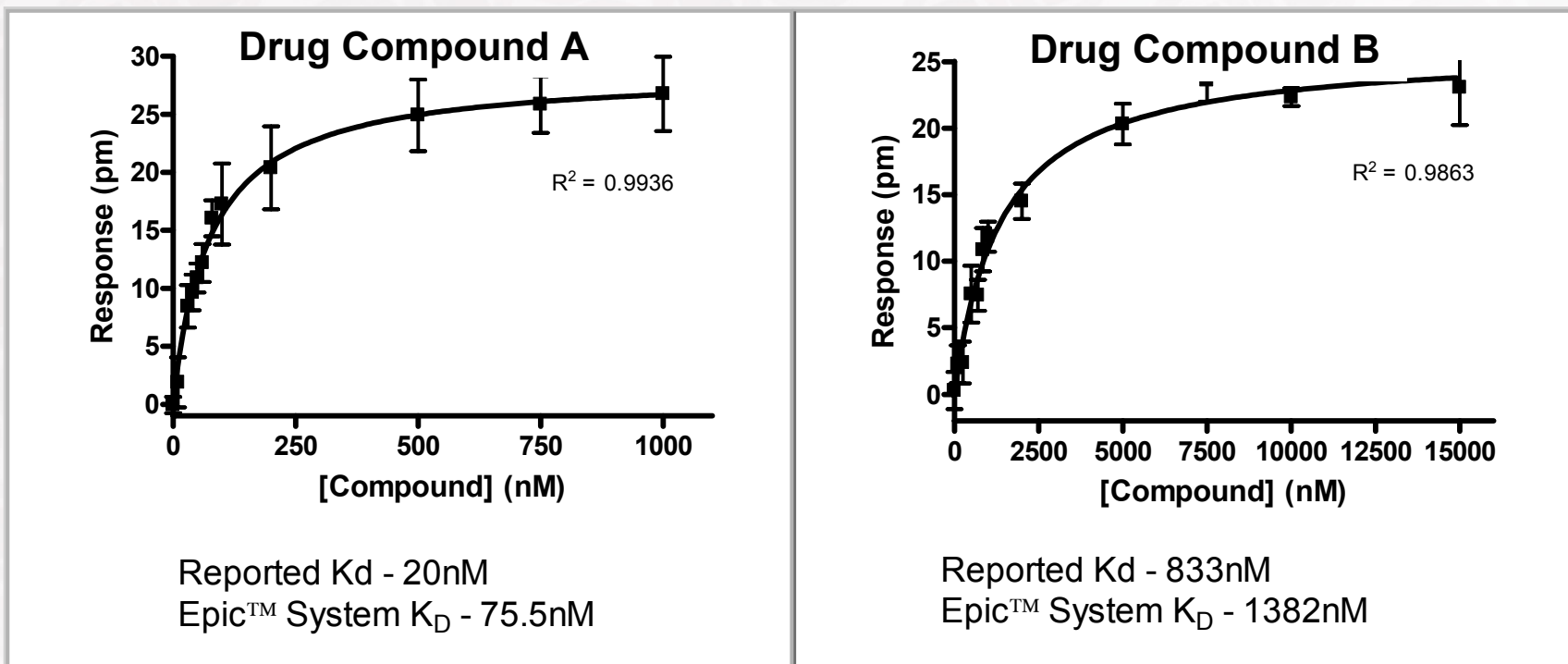
$$K_D = 60\text{nM} \pm 12\text{nM}$$

$$K_D = 102\text{nM} \pm 9\text{nM}$$



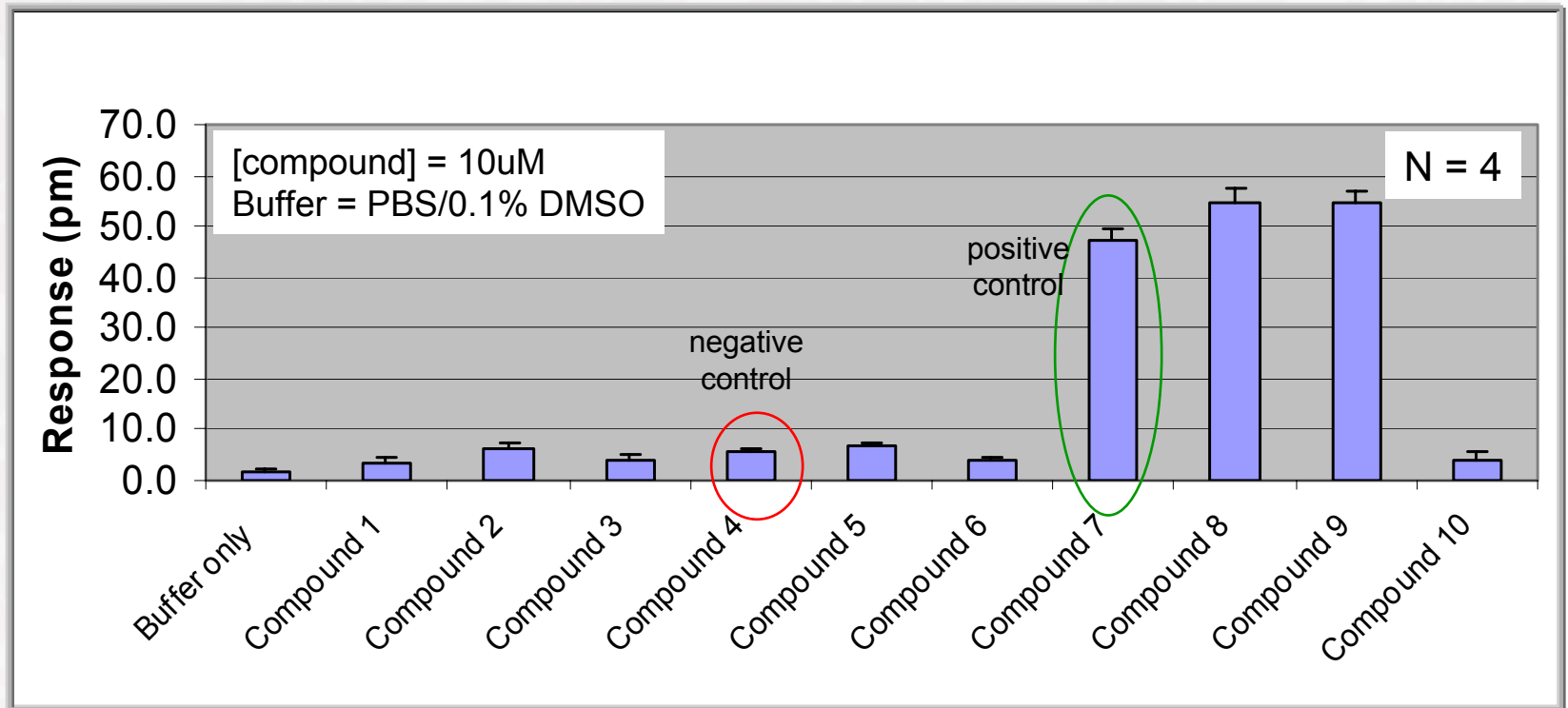
Direct Bind Kinase Assay

- Compound (~350 Da) binding to immobilized kinase (45 kDa):



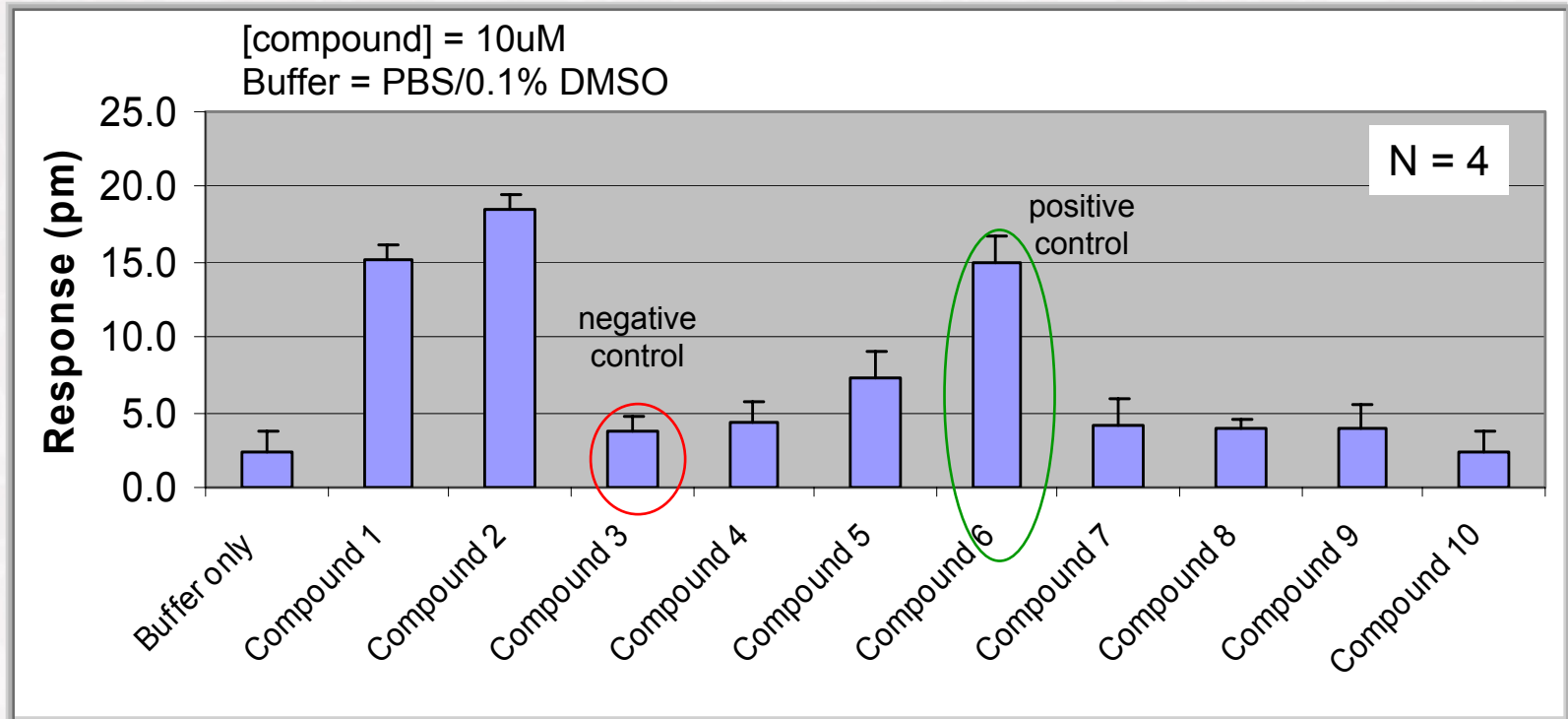
Conclusions: Good signal levels and low CVs are observed.
Estimated K_d values are consistent with reported values

Protease Direct Bind Assay Compound Screen



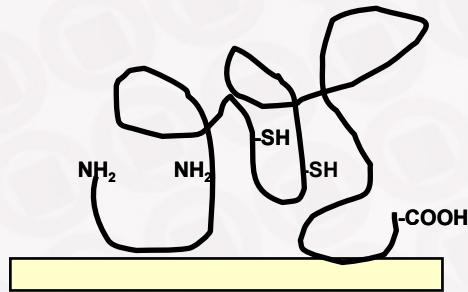
- Compound MW range:320Da – 800Da
- Compounds 7 (positive control), 8 and 9 show binding.
- The negative control (compound 4) shows no binding.

Protease Direct Bind Assay Compound Screen



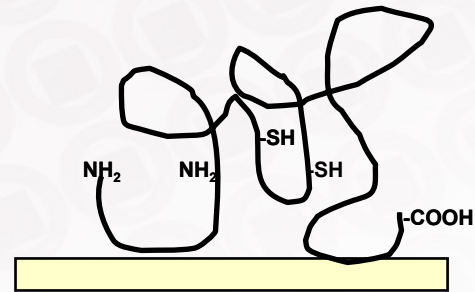
- Compound MW range:320Da – 800Da
- Compounds 1, 2, 6 (positive control) show binding.
- The negative control (compound 3) shows no binding

Protease Functional Assays

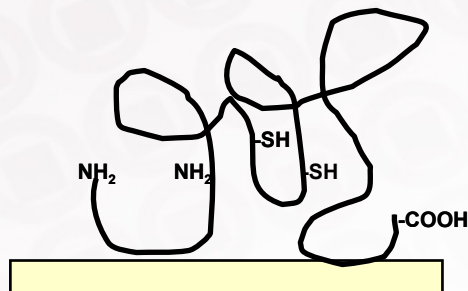


Immobilized protein substrate

protease
+ inhibitor

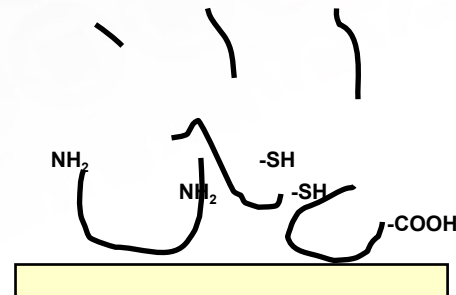


No digestion/loss of protein
No change in signal



Immobilized protein substrate

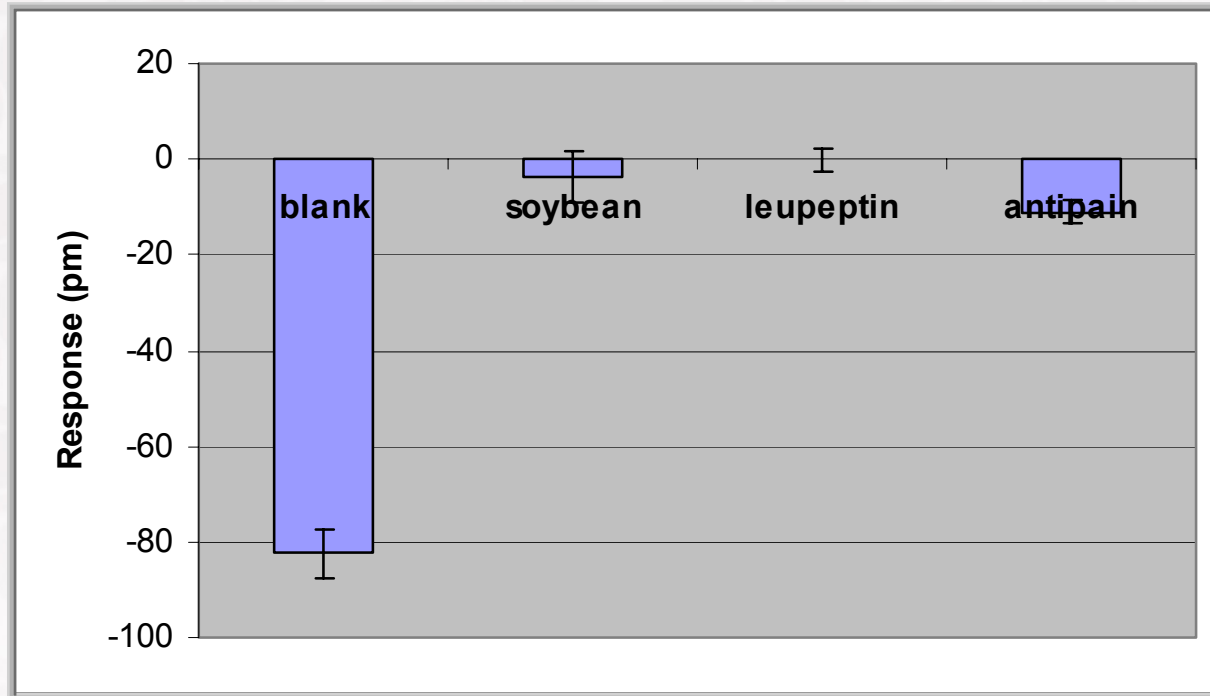
protease



Digestion/loss of protein
Decrease in signal

Protease Functional Assay Inhibitor Screen

- Immobilize human serum albumin
- Add trypsin + inhibitor

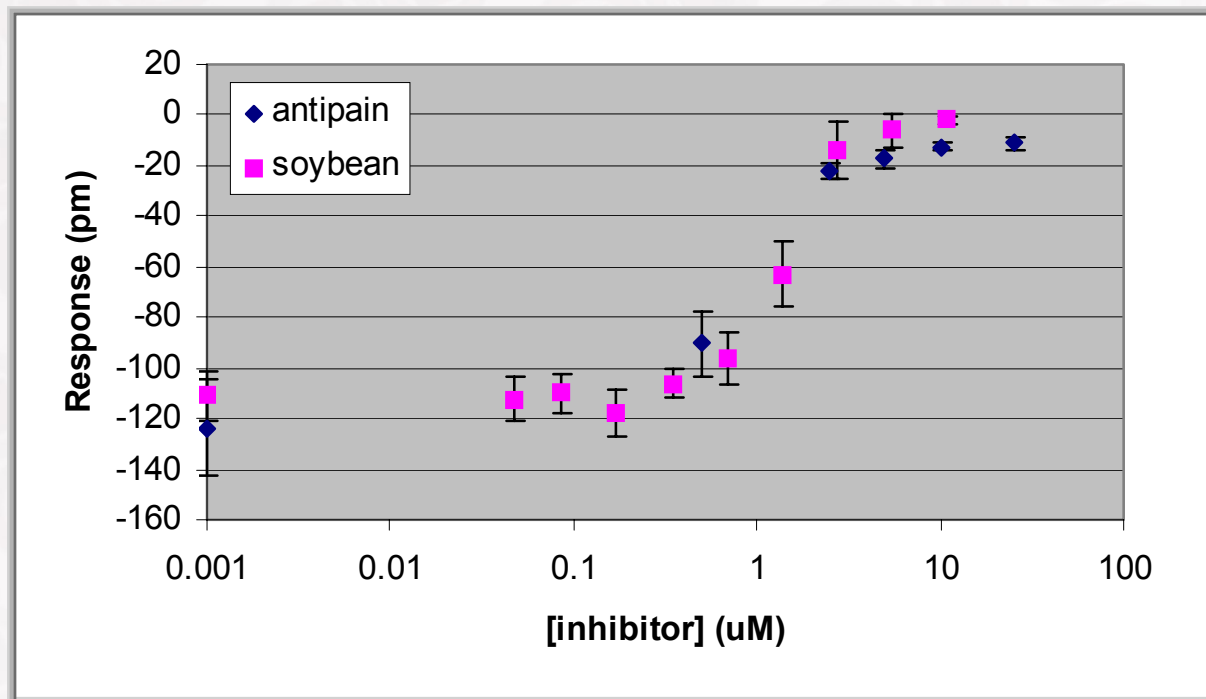


Conclusion: Trypsin inhibitors can be easily detected.

Protease Functional Assay

Inhibition of Trypsin Activity

HSA: 50ug/ml in pH 5.0 acetate buffer
Trypsin: 10units/well in 1X PBS



Antipain

IC₅₀ (Epic) = 0.85uM

IC₅₀ (Lit) = 0.43uM

Soybean

IC₅₀ (Epic) = 2.1uM

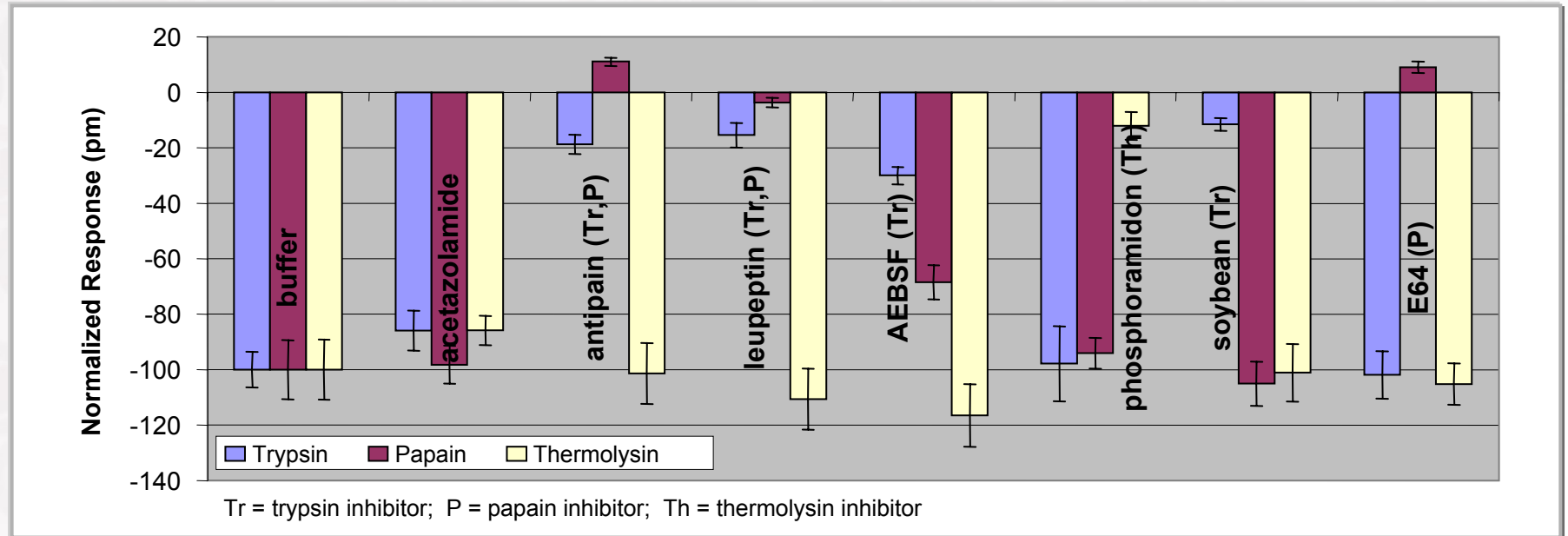
IC₅₀ (Lit) = NR

Conclusion: Trypsin activity is inhibited in a dose-dependent manner

Protease Functional Assay

Cross Screen of Inhibitors; Z's > 0.50

- Immobilize protein substrate (carbonic anhydrase)
- Add protease + inhibitor



- Inhibitor concentrations were 10uM except for AEBSF (400uM), E64 (5uM) and phosphoramidon (100uM)
- Amounts of protease/well were 20U (trypsin); 0.05U (thermolysin); 0.25U (papain)
- Digestion time was 30 minutes

Epic™ Biochemical Assays: Key Points

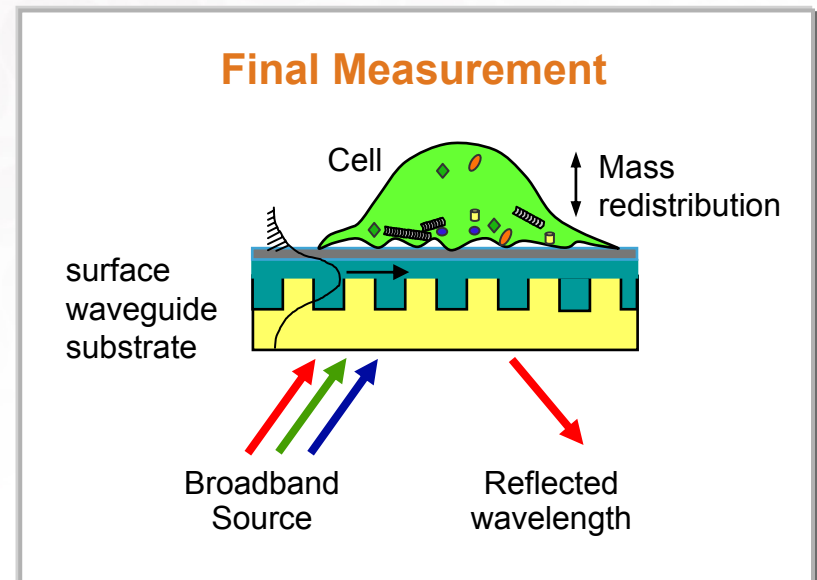
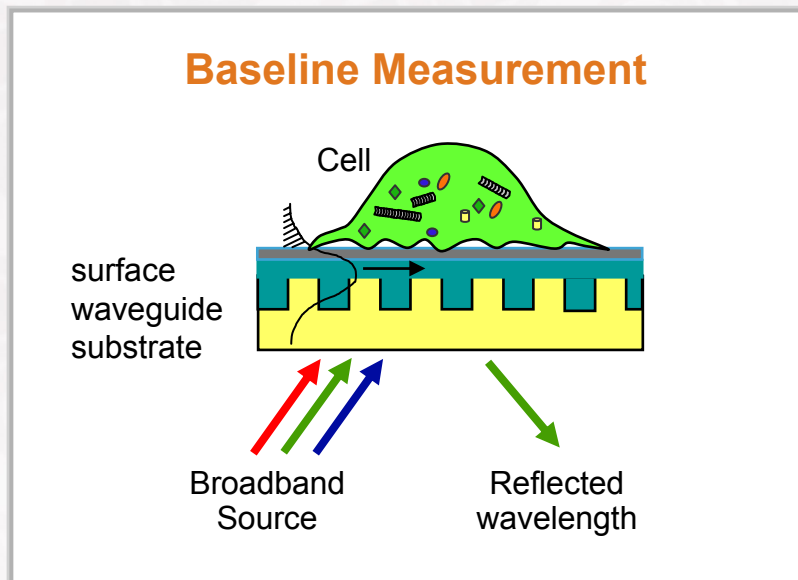
- We have demonstrated ability to measure small molecule binding for compounds as small as 157 Da in HTS
- The measured biomolecular affinities show good agreement with expected values
 - Dose response curves
 - Specificity
- The Epic™ System is a versatile tool for biochemical assays
 - Yes/No binding / Affinity ranking
 - Direct bind / Functional
 - HTS primary screening

CELL-BASED ASSAYS

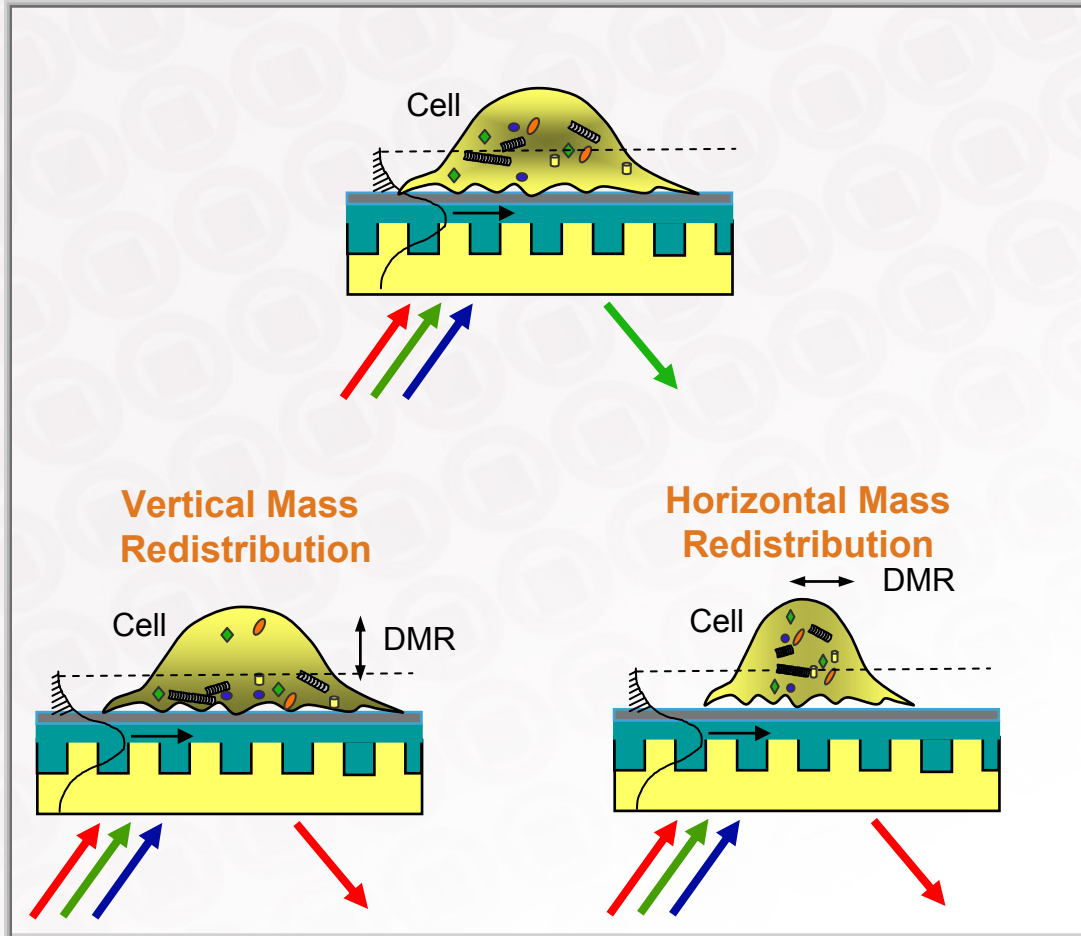


Operating Principle: Cell-based Assays

- Measures changes in local index of refraction from mass redistribution within a thin layer of the cell monolayer
- Change in index manifest by a shift in resonant wavelength
- Sensitive to the first ~150nm from the sensor surface



Dynamic Mass Redistribution (DMR)



Events that Induce DMR

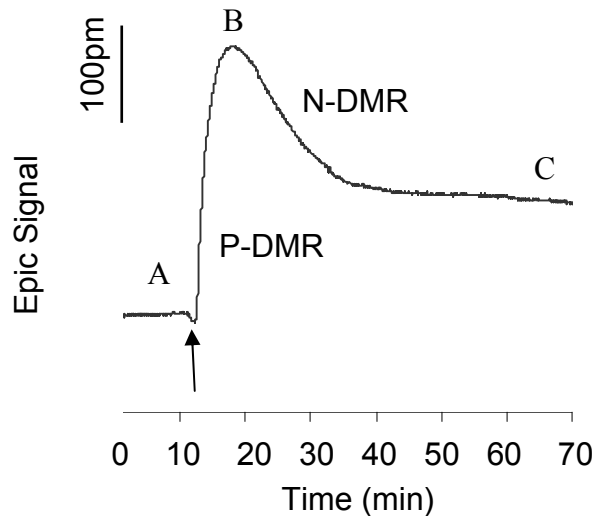
- Protein recruitment
- Endocytosis and recycling
- Exocytosis
- Apoptosis
- Cytoskeletal rearrangement

DMR Optical Response Theory

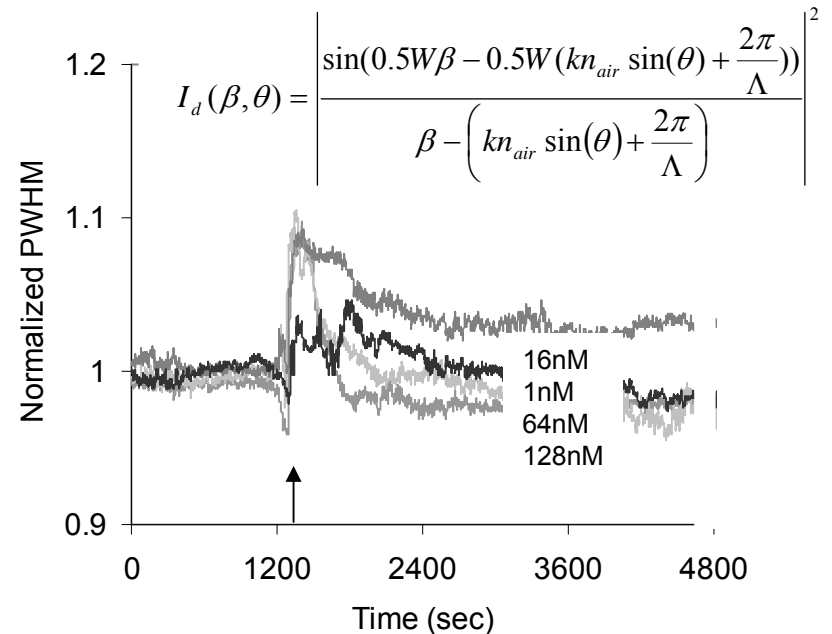
- The DMR optical response to cellular stimulation is a function of only two terms:
 - Sensing distance
 - Mass concentration

Vertical Mass Redistribution

$$Signal = a\Delta C_i(t) \sum \left(e^{-\frac{z_i}{\Delta Z_c}} - e^{-\frac{z_{i+1}}{\Delta Z_c}} \right)$$

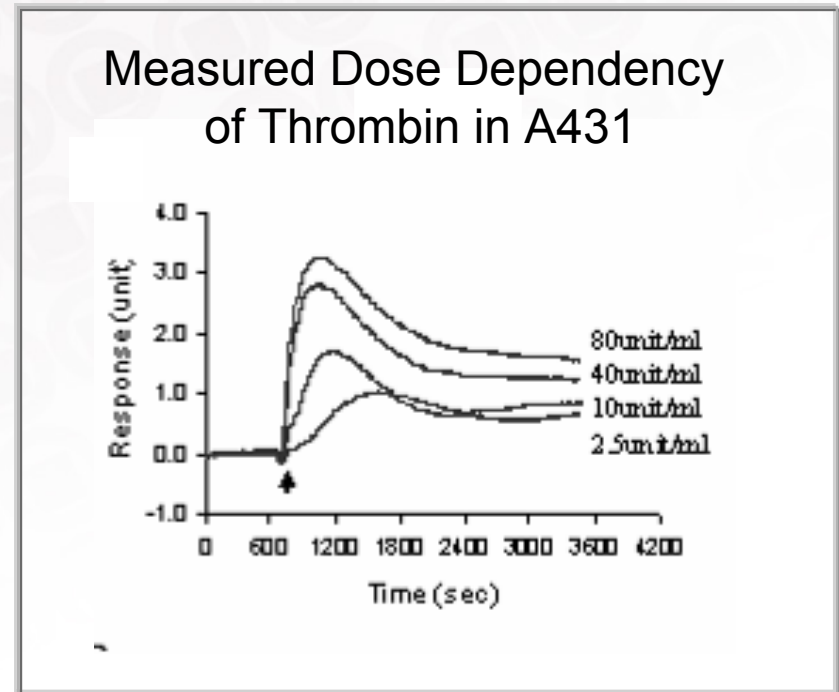
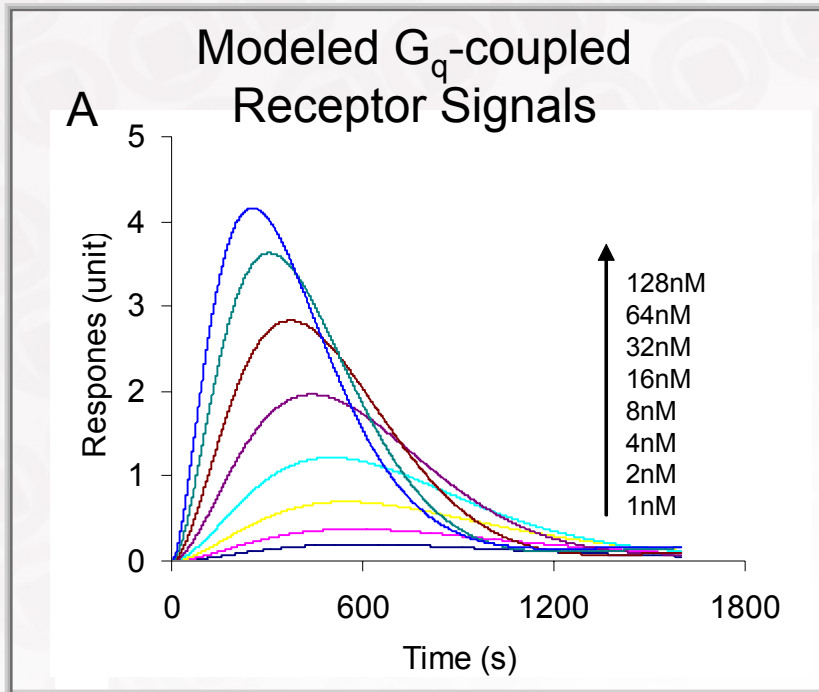


Horizontal Mass Redistribution



Numerical Modeling: G_q -Coupled Receptor Signals

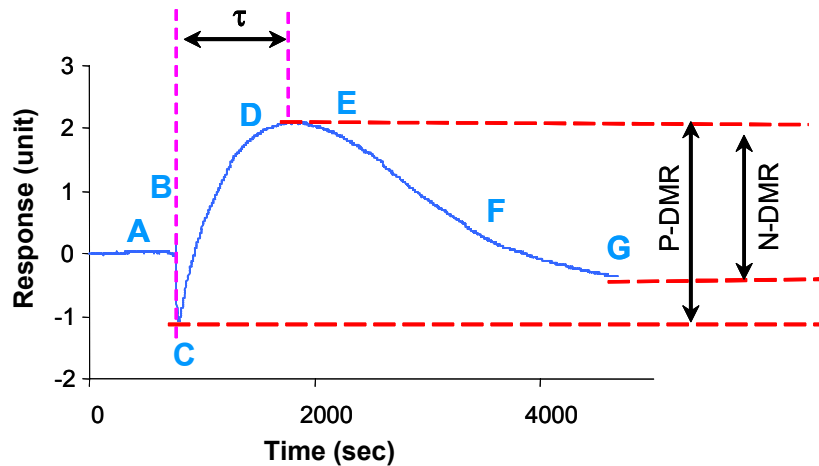
- Model based on protein trafficking and mass transport equations
- Numerical modeling shows good agreement with experimental results



Fang, Y., et al. (2006) *Biophys. J.*, 91, 1925-1940.

Epic™ Whole Cell Assays

Epidermal Growth Factor Receptor (EGFR) in A431 Cells



KEY:

A: Equilibration with buffer

B: Compound addition

B to C: bulk index change

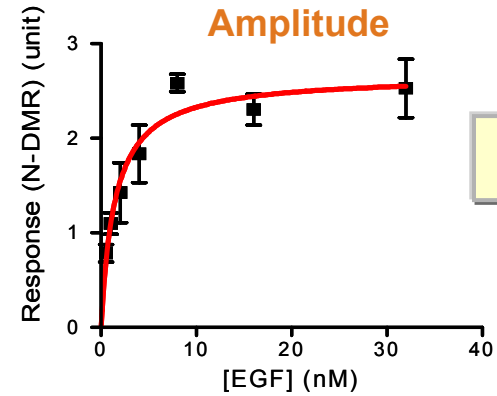
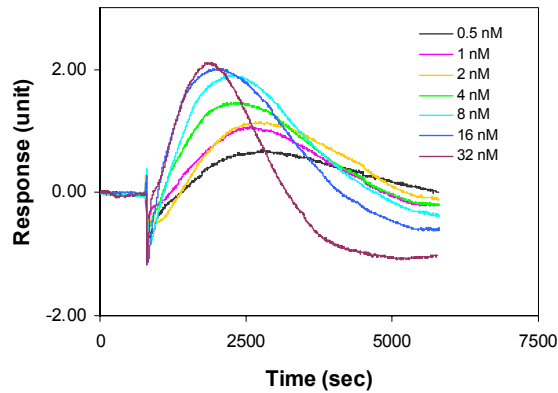
C to D: increased mass within the sensing volume

D to E: steady transition state

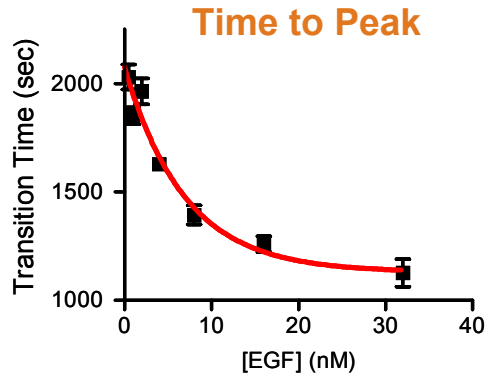
E to G: decreased mass within the sensing volume

Major Events	Time scale	Signal
Ligand binding	sec to minutes	negligible
Receptor activation	ms to sec	negligible
Signaling propagation	ms to sec	negligible
Intracellular recruitment	sec to minutes	large, positive
Endocytosis	minutes to half hour	large, negative
Receptor Recycling	~15min after endocytosis	small, positive
Cell morphological changes	minutes	large, pos or neg

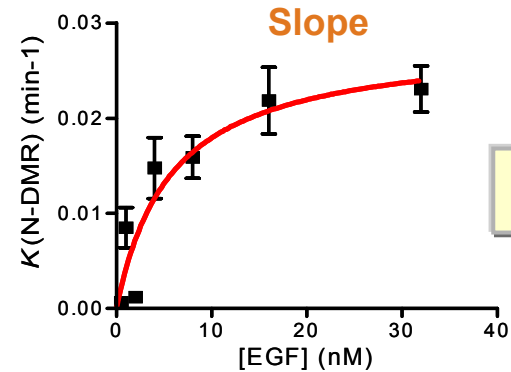
Epic™ Whole Cell Assays: EC₅₀ Estimation Epidermal Growth Factor Receptor (EGFR) Assay



EC₅₀ ~1.4nM



EC₅₀ ~4 nM

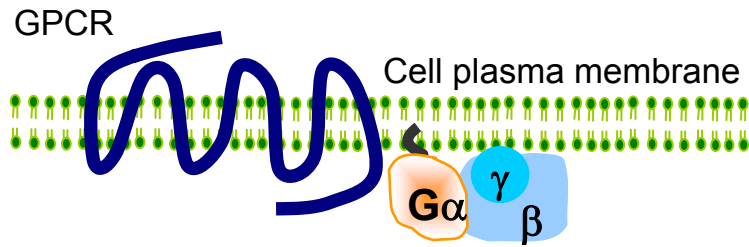


EC₅₀ ~5.7nM

Conclusion: The response is EGF dose dependent and saturable.

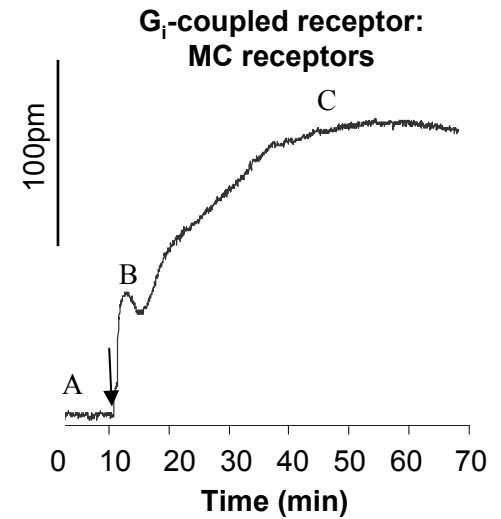
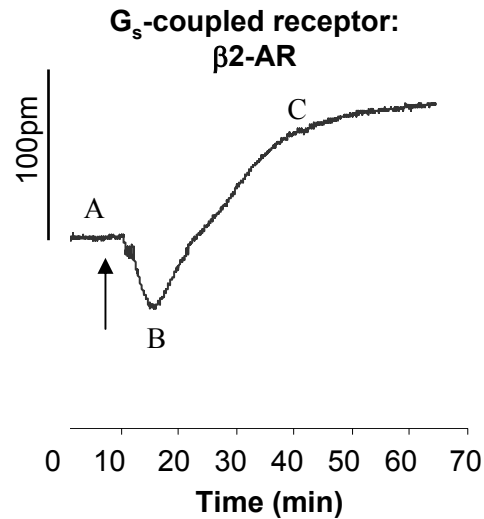
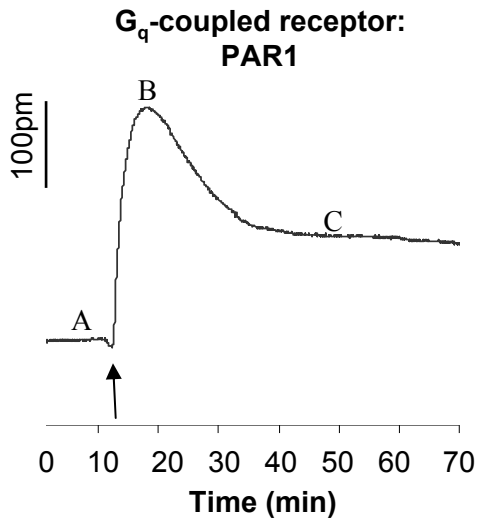
Epic™ System is Applicable to All Three Classes of GPCRs

Example Profiles for A431 Cells



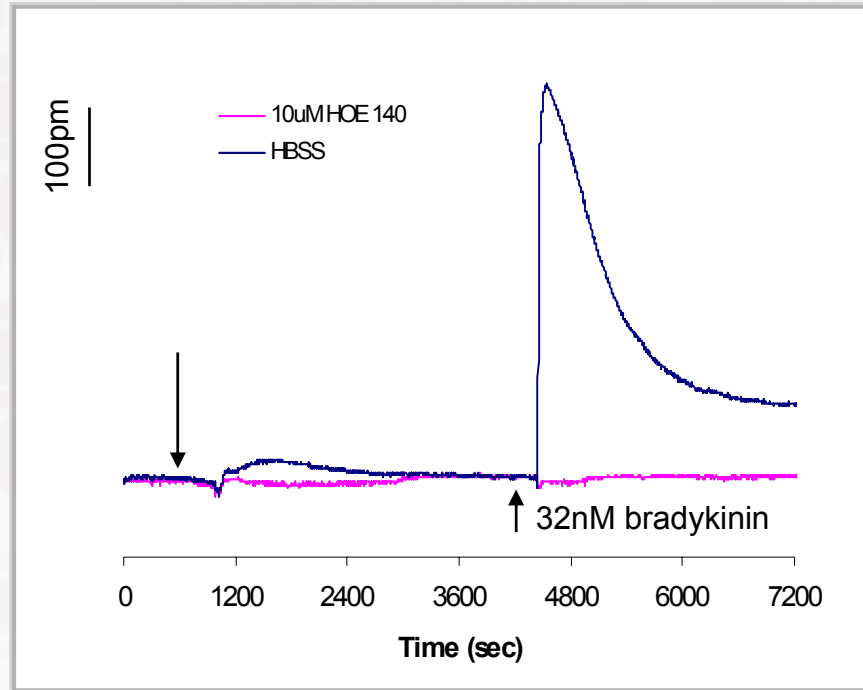
Three classes of GPCRs, depending on the G protein to which the receptor is coupled:

- $G_{\alpha q}$ – release of Ca^{2+}
- $G_{\alpha s}$ – accumulation of cAMP
- $G_{\alpha i}$ – inhibiting cAMP generation



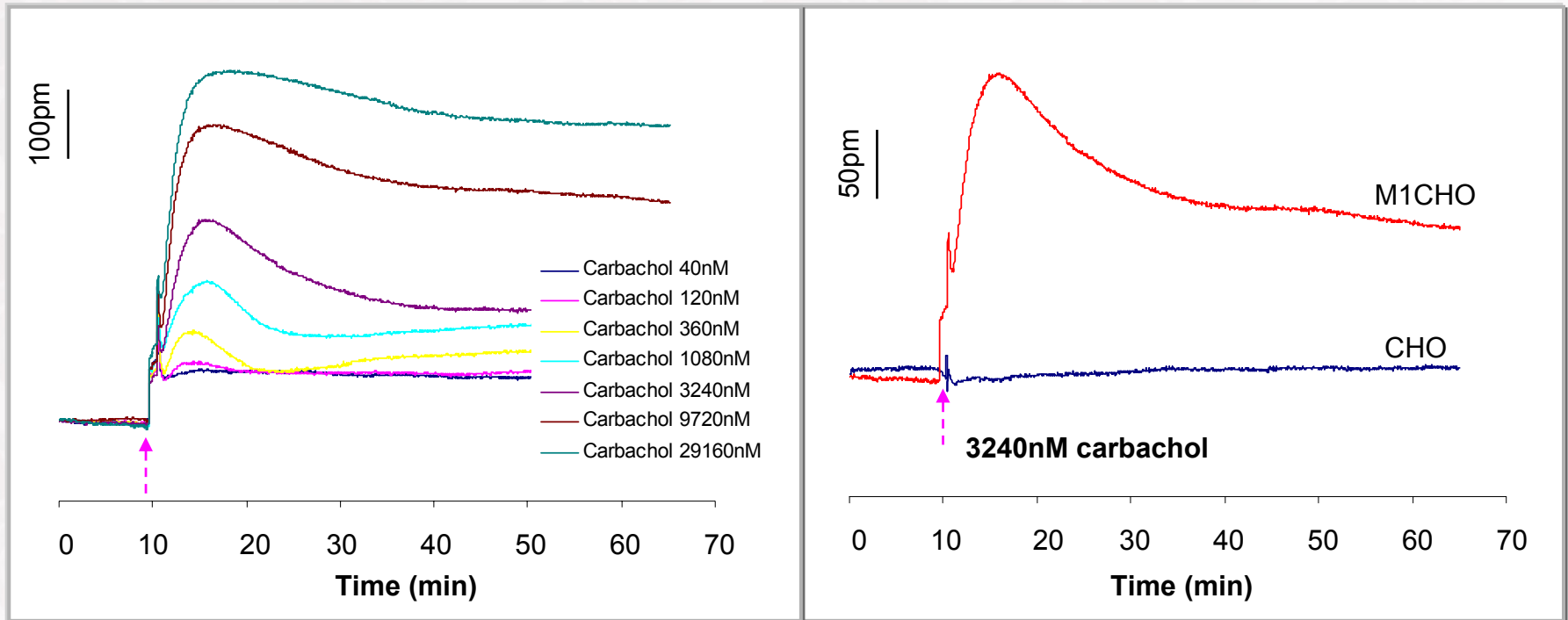
G_q-Coupled Receptors With Epic™ System

Specificity of Bradykinin B2 Receptor Response in A431



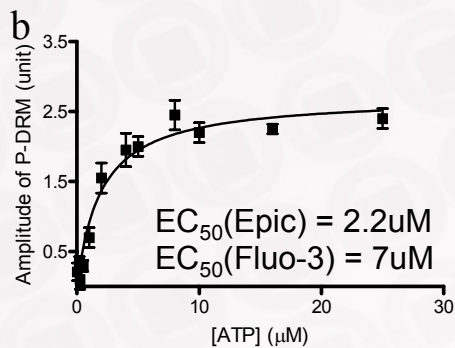
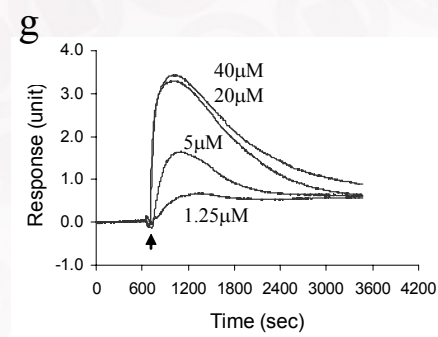
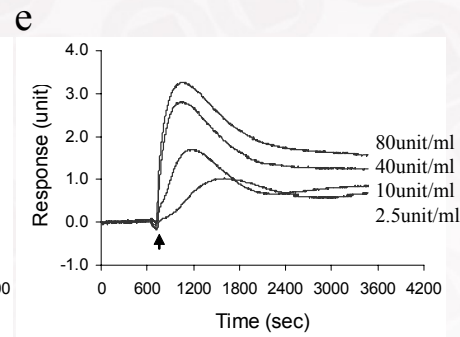
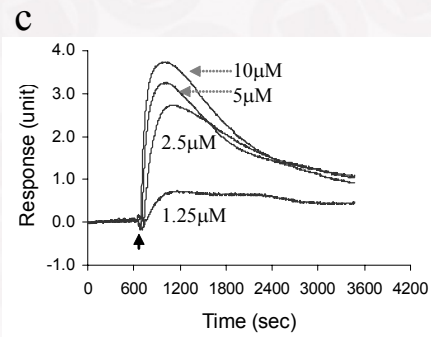
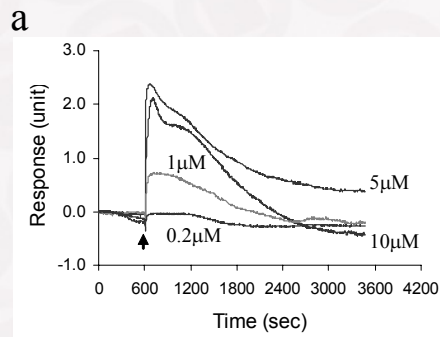
- HOE140 – a B2-specific antagonist – dose-dependently inhibits the bradykinin-induced signals

Whole Cell GPCR Assays: Specificity of Response Profiles

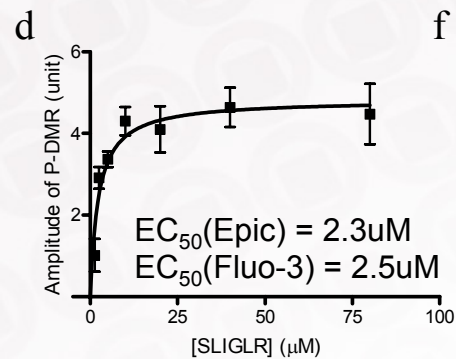


- Carbachol is an agonist for both muscarinic and nicotinic receptors
- Carbachol dose-dependently mediates a Gq-type signal in M1CHO cells, but not in CHO cells.

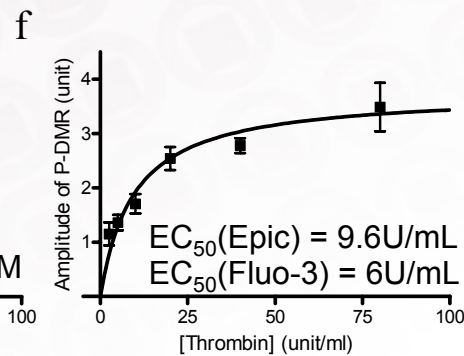
Profiling Ligands for *Endogenous* G_q-Coupled Receptors in A431 Cells



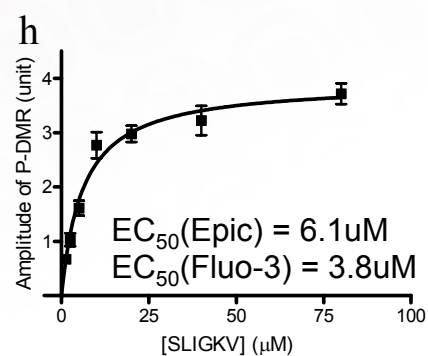
P2Y receptors



PAR2 receptor

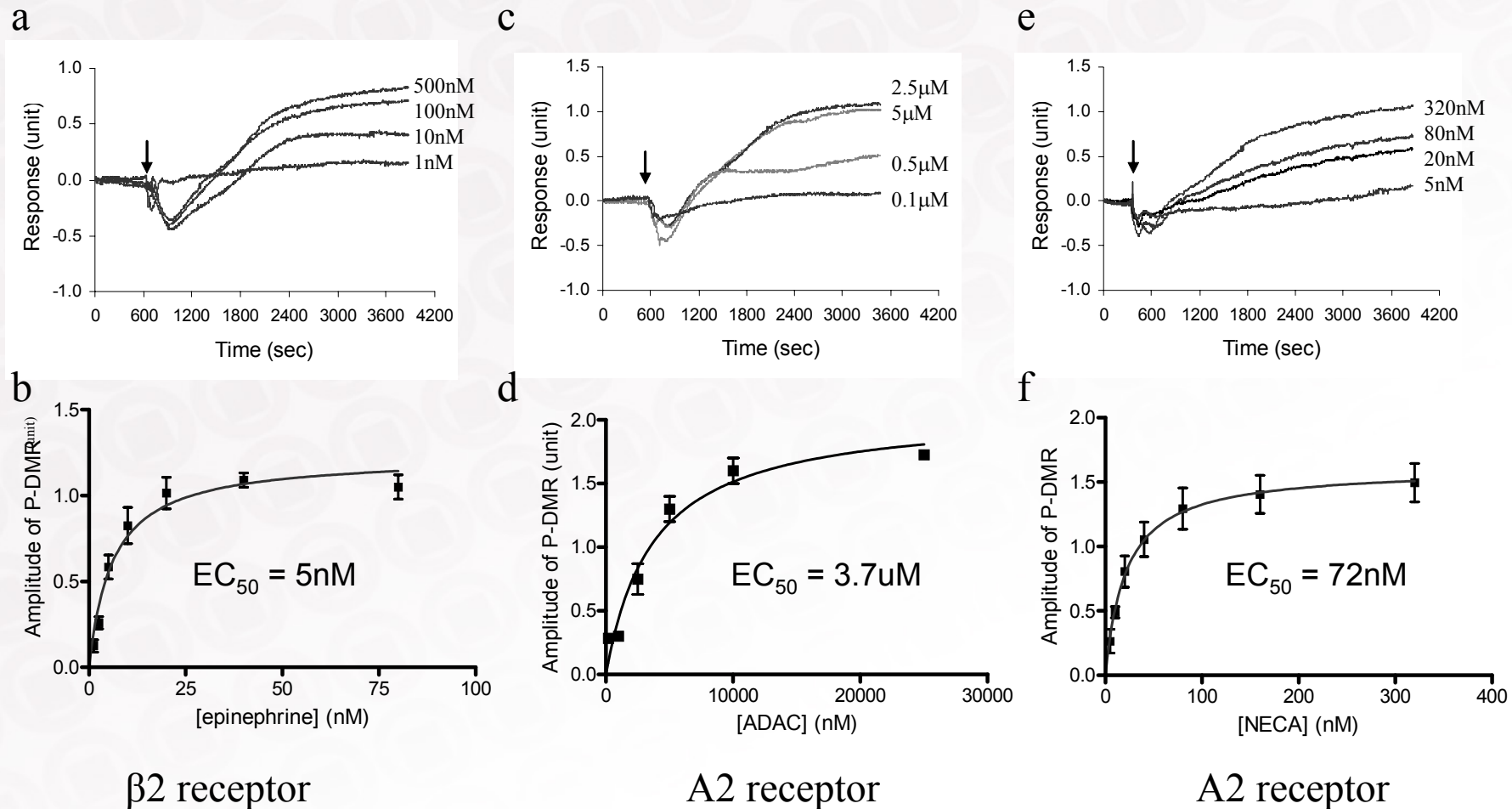


PAR1 receptor



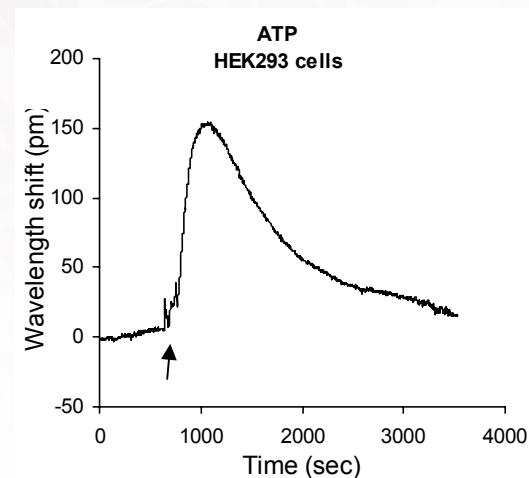
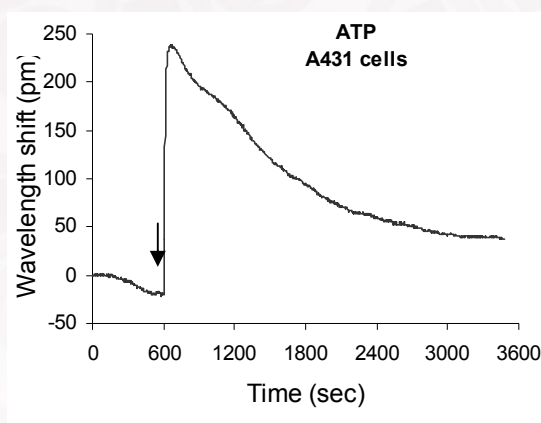
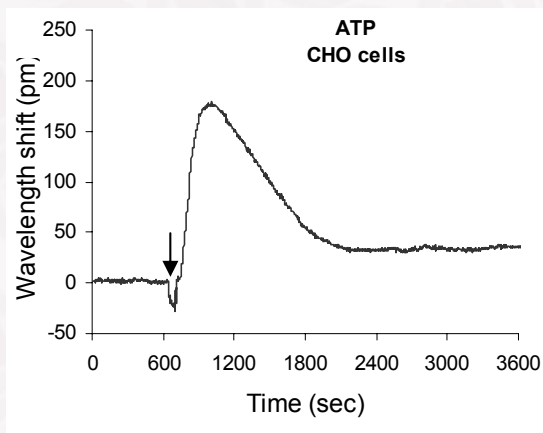
PAR2 receptor

Profiling Ligands for *Endogenous* G_s-Coupled Receptors in A431 Cells



Endogenous Receptor Profiling Across Cell Lines

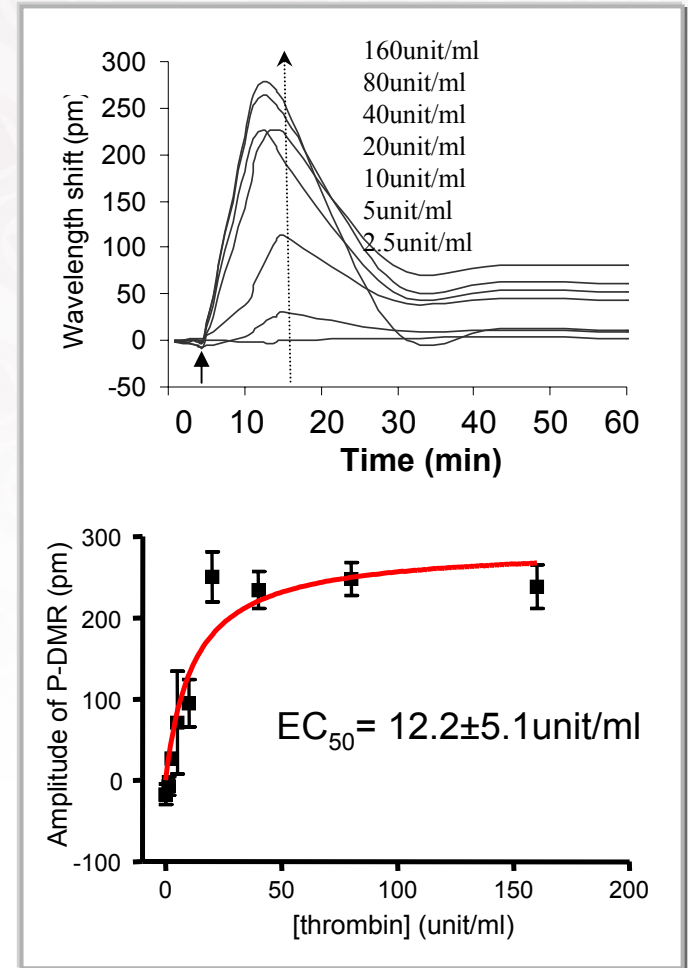
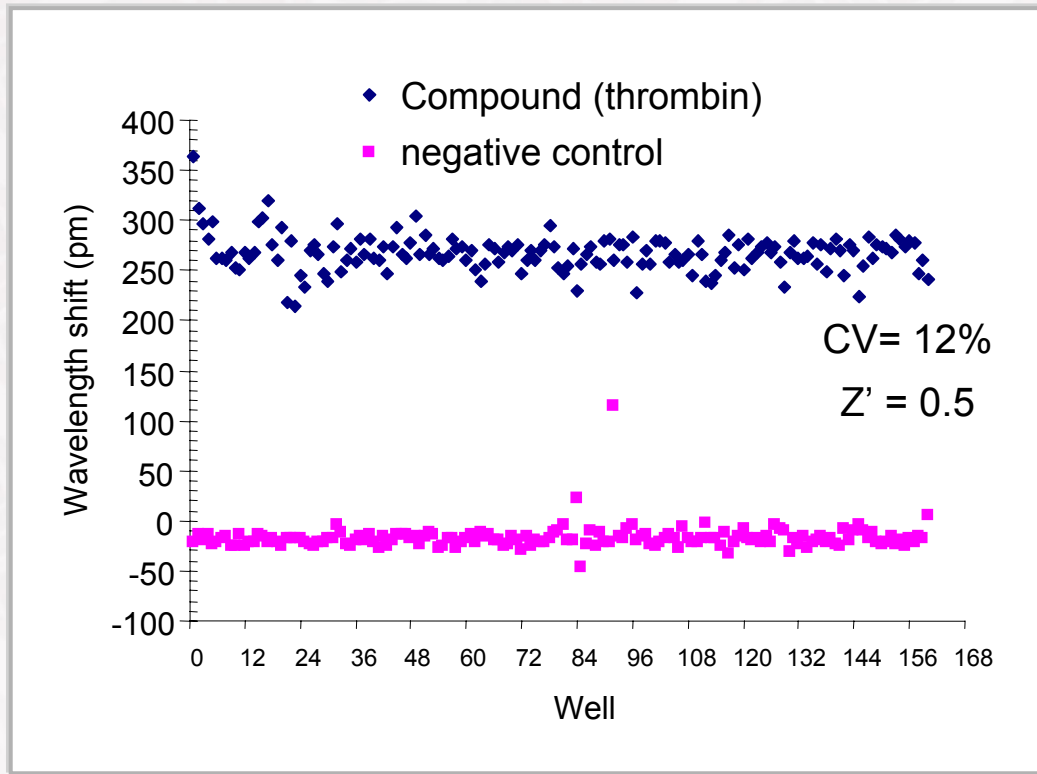
- Panning endogenous P2Y receptors in multiple cell lines
 - Efficacy rank order to determine the subfamily member receptor(s) that dominate the DMR signal
 - Difference in the optical signature reflects the signaling network interaction mediated through P2Y receptors



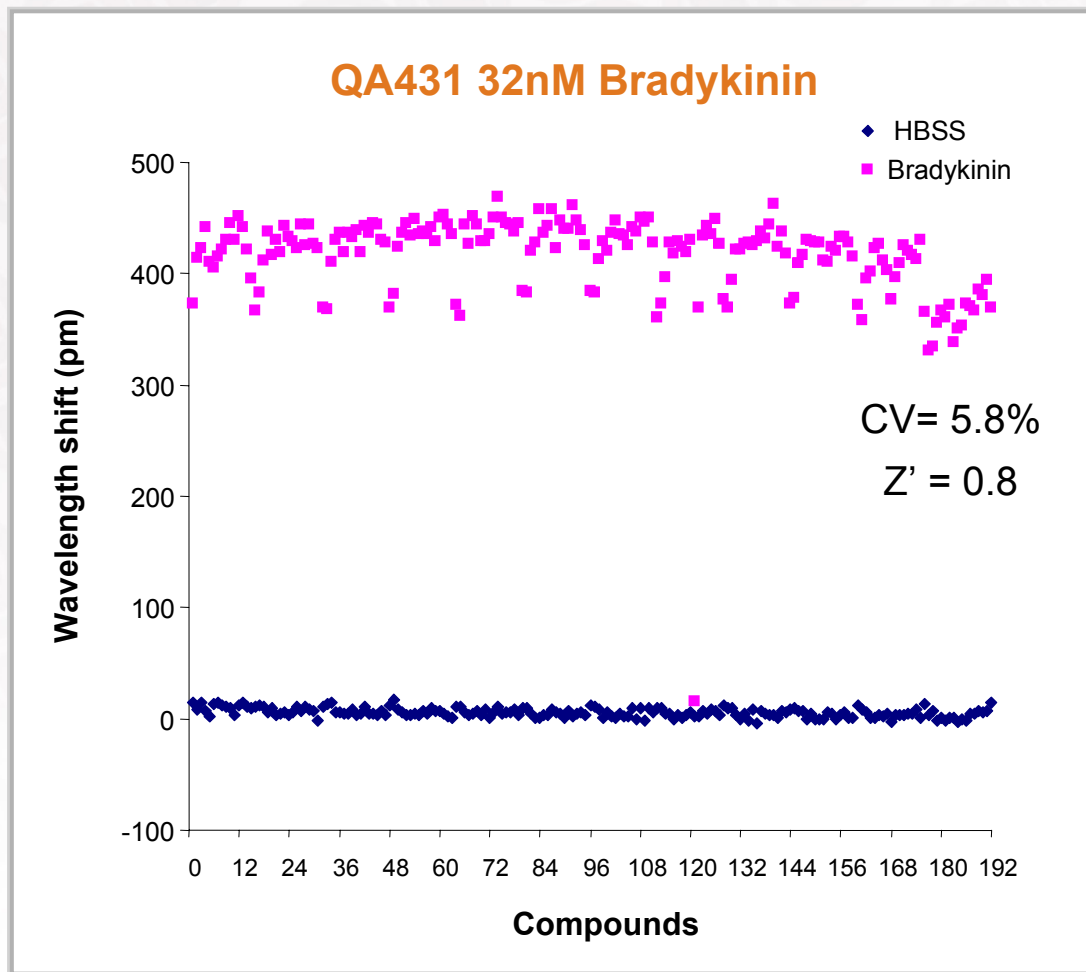
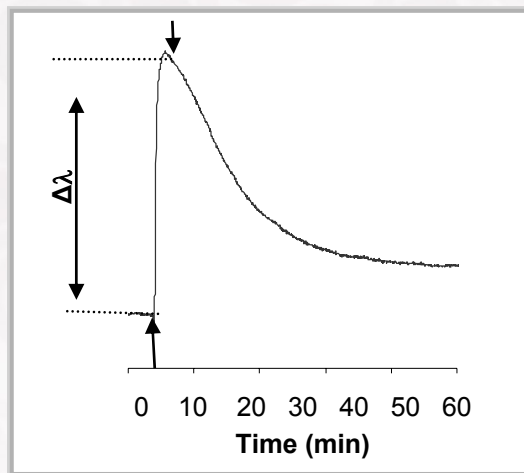
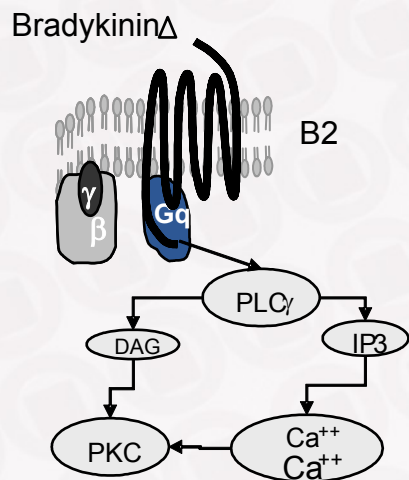
EC₅₀

	ATP	ADP	ATP _γ S	UTP	UDP	UTP _γ S
A431	252nM	11307nM	400nM	247nM	7906nM	173nM
CHO	394nM	3590nM	374nM	475nM	8783nM	316nM
HEK	5μM	2μM	40μM	92μM	Not active	Not active

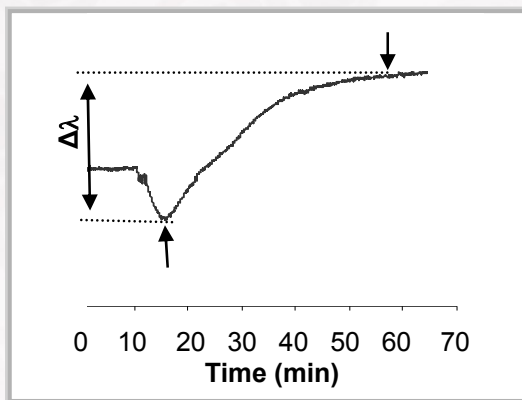
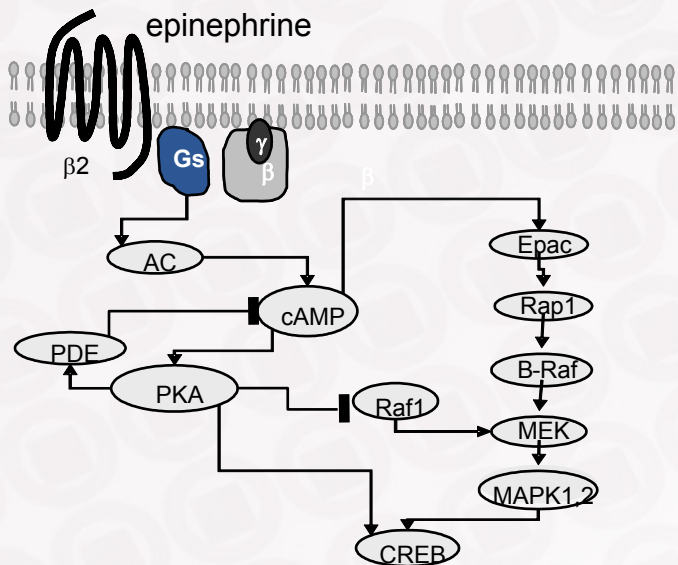
HTS G_q -Coupled Receptors With Epic™ System: Endogenous Protease-Activated Receptor 1 in CHO-K1



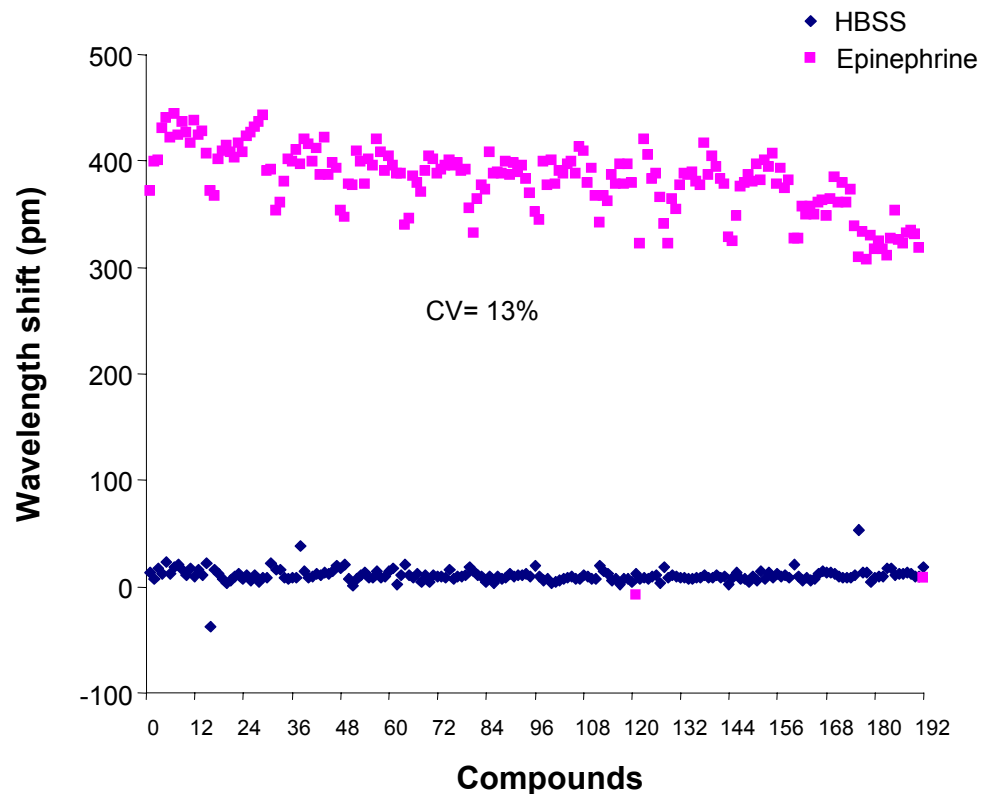
HTS G_q-Coupled Receptors With Epic™ System: Endogenous Bradykinin B2 Receptor in A431



HTS G_s-Coupled Receptors With Epic™ System: Endogenous β 2 Adrenergic Receptor in A431



QA431 25nM Epinephrine



Cell Lines and Assays Developed

Cell Assays	Cell Systems
GPCR assays	A431 CHO M1CHO HEK 293 Cos7
EGFR assays	A431
Lipid signaling	A431 HeLa
Cytotoxicity	A431 CHO
Proliferation assays	CHO A431
Cell Adhesion	CHO A431
Kinases	A431 Jurkat
ROS signaling	A431

Cell-Based Assay Summary

- High sensitivity of Epic™ System enables direct measurement of endogenous receptor response
 - More in-vivo like response
 - No licenses required for proprietary receptors
- Suitable for primary cells and overexpressed cell lines
- Screen GPCRs coupled via different types of G proteins (G_s , G_i , G_q)
- Platform can be used for both high content and high throughput screening applications
 - Endpoint or kinetic information
 - Enables studies of systems biology and pharmacology

Presentations at SBS

Posters: Tues 9/19, 12:30 – 2:30

- **(P7030)** Label-free Profiling of Ligands for Endogenous GPCRs (Joydeep Lahiri)
- **(P7154)** Whole Cell GPCR Assays using Corning® Epic™ Label-free System (Gary Li)
- **(P7155)** Analysis of Small Molecule/Protein Interactions Using Corning® Epic™ System (Arron Xu)
- **(P7064)** Analysis of Small Molecule/Enzyme Interactions Using the Corning® Epic™ System (Tony Frutos, Christian Bergsdorf – Schering AG)
- **(P7124)** Evaluation of the Corning® Epic™ System for label-free function GPCR assays (Kathryn Dodgson, AZ)

Podium Talk: Wed 9/20, 4:25pm Room 6B

- Phosphorylation Profiling: High-Throughput Label-Free Detection of Protein-Protein Interactions with the Epic™ System (Dr. Meng Wu, JHU)

Summary



- Label-Free Detection in High Throughput
 - 384-well microplate, 40,000 wells/8 hours
- Broad Applicability
 - Biochemical and cell-based assays
 - Direct binding and functional assays
- High Sensitivity
 - Small molecule
 - Endogenous receptors
- Delivered by a world leader in life sciences, photonics, & materials science