



BellBrook
LABS

12th Annual SBS Conference

**Emerging Covalent Regulatory Families
and Phosphodiesterases**

Robert Lowery
President & CEO

SCREENING SIMPLIFIED. DRUG DISCOVERY MULTIPLIED.

Note: Since writing this presentation Transcreener names have changed to better reflect their universality.

- Transcreener KINASE Assay is now Transcreener ADP Assay
- Transcreener PDE Assay is now Transcreener AMP/GMP Assay
- Transcreener UGT-488 Assay is now Transcreener UDP Assay

Summary

- Group transfer reactions, covalent regulation and the Transcreener™ HTS Assay Platform:

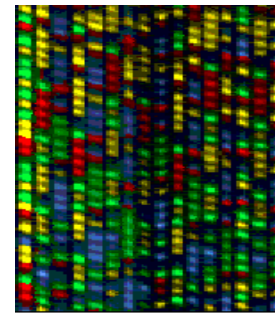
Phosphorylation, Glycosylation, Methylation, and Sulfation

- Transcreener™ AMP detection:

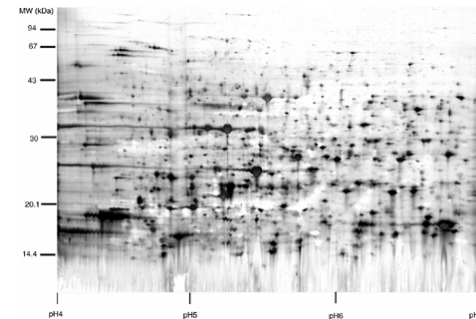
cAMP/cGMP phosphodiesterases

Posttranslational modification increases proteome complexity

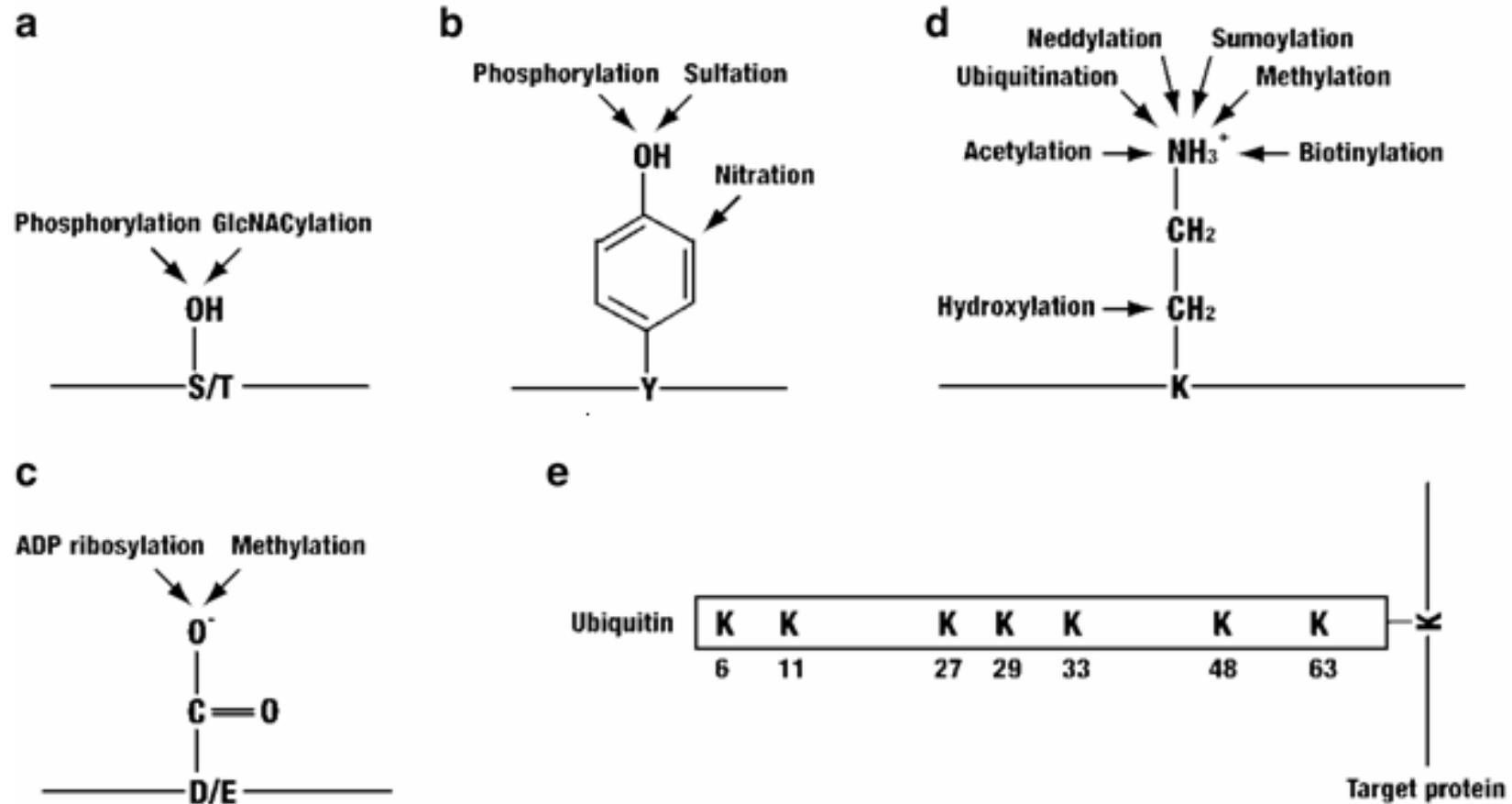
- **Genome: 30,000**



- **Proteome: 3,000,000**

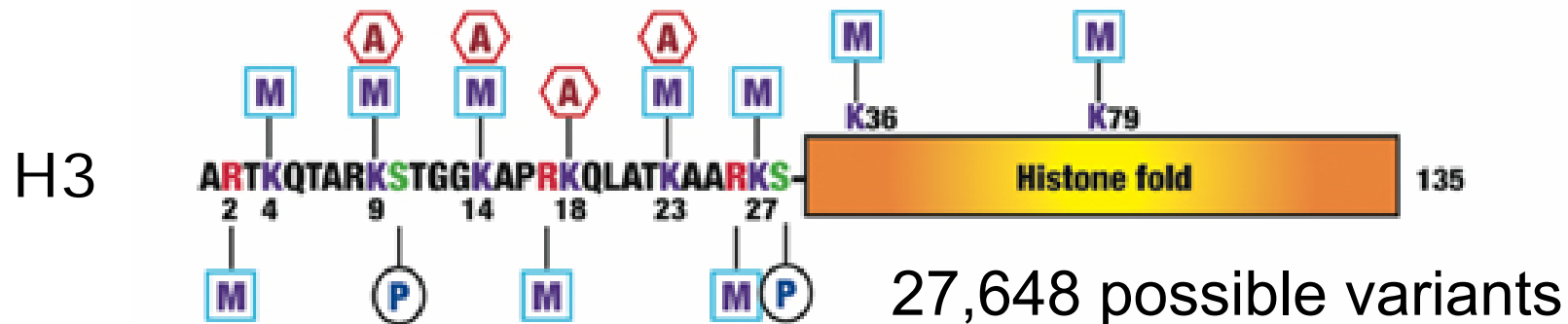
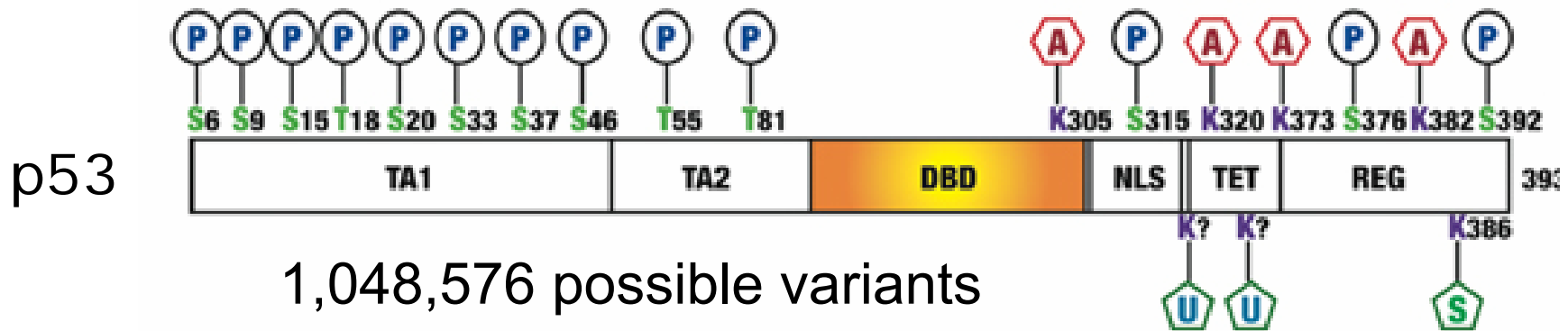


Sites of covalent modification



Multisite protein modification and intramolecular signaling.
 Xiang-Jiao Yang, (2005) *Oncogene* 24, 16553-1662

P53 and Histone H3 modifications

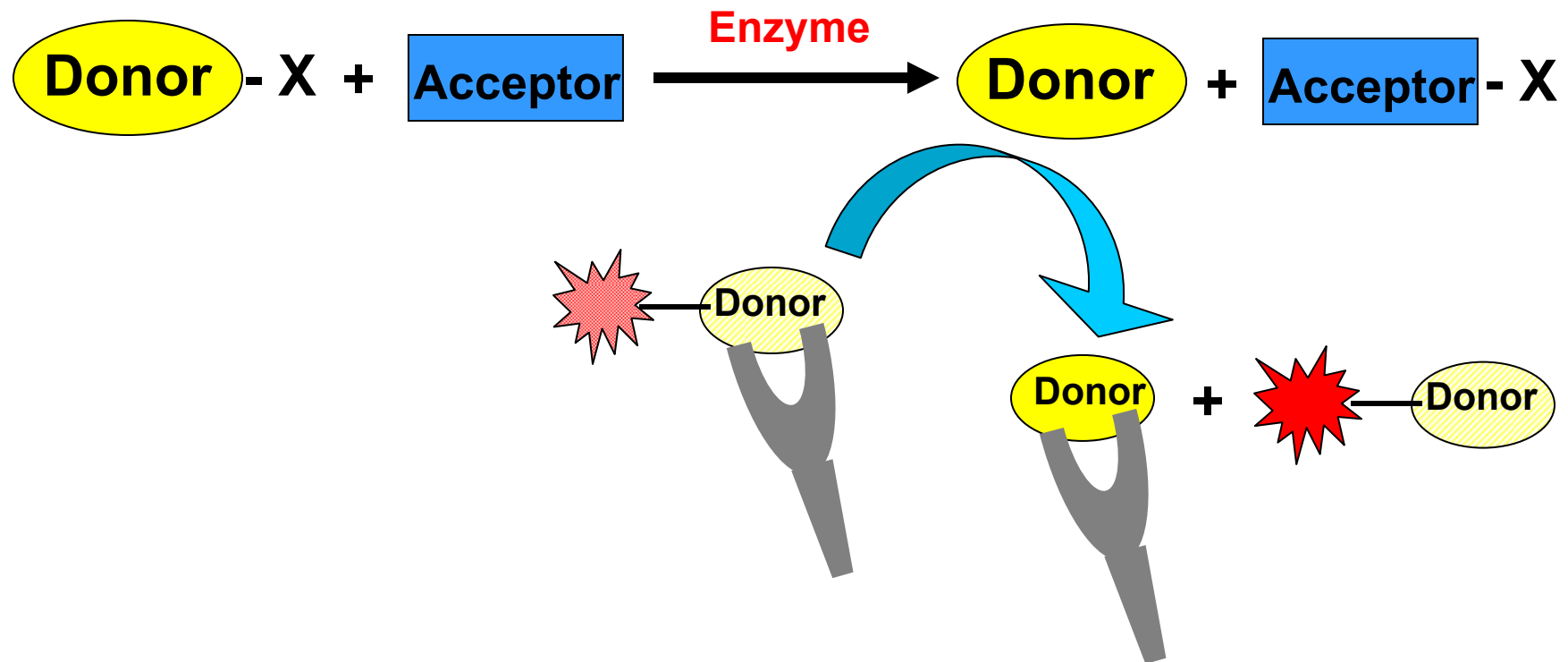


Multisite protein modification and intramolecular signaling.
 Xiang-Jiao Yang, (2005) Oncogene 24, 16553-1662

Group Transfer Enzymes Control Diverse Biomolecules and Disease Pathways

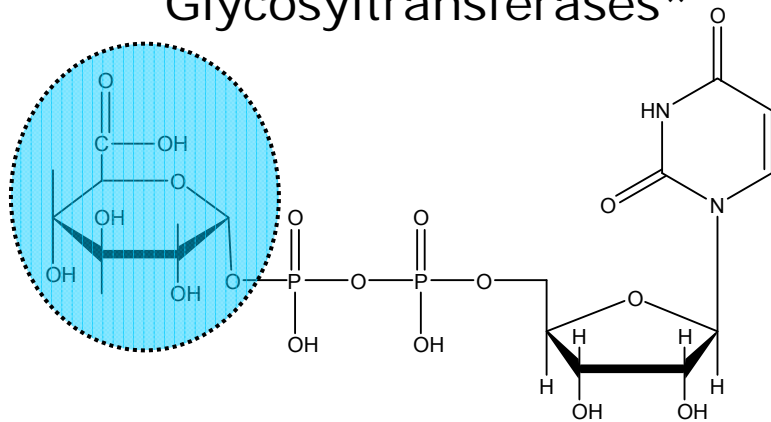
Target Family	Substrates	Disease Areas
Kinases (>650)	Proteins, Lipids, Carbohydrates, Nucleic Acids	Cancers, Inflammation, Diabetes, Cardiovascular, Neurological
Glycosyltransferases (>200)	Proteins, proteoglycans, <i>small molecules</i>	Drug Metabolism, Cancer, Antimicrobials
Sulfotransferases (>50)	Proteins, proteoglycans, <i>small molecules</i>	Drug Metabolism, Cancers, Cardiovascular, Neurological
Methyltransferases (>50)	Proteins, DNA, <i>small molecules</i>	Drug Metabolism, Cancers, Neurological
Acetyltransferases (>10)	Proteins, DNA, <i>small molecules</i>	Drug Metabolism, Cancers

Transcreener™ Assay Platform: Immunodetection of Invariant Reaction Products

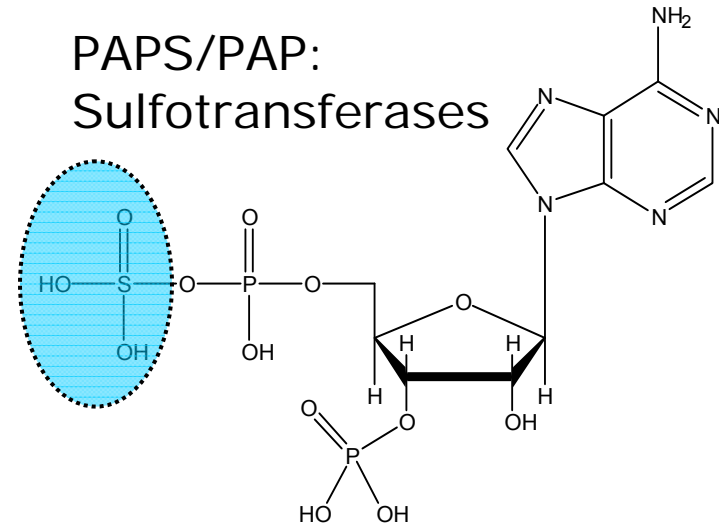


Transcreener™ Platform: Selective Nucleotide Detection

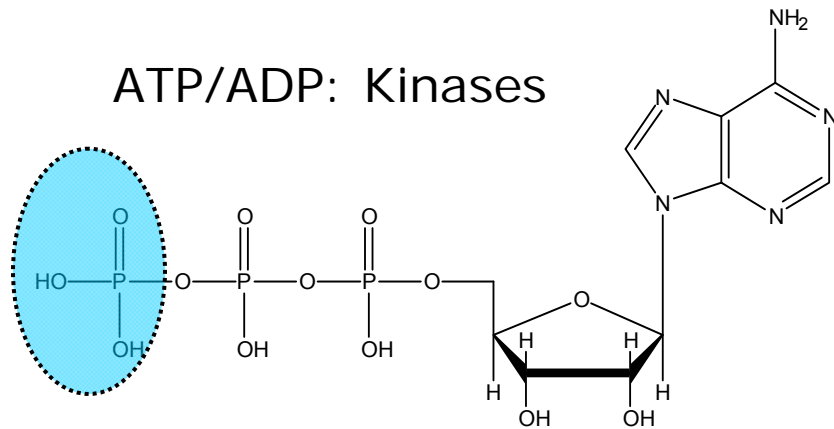
UDPGA/UDP:
Glycosyltransferases*



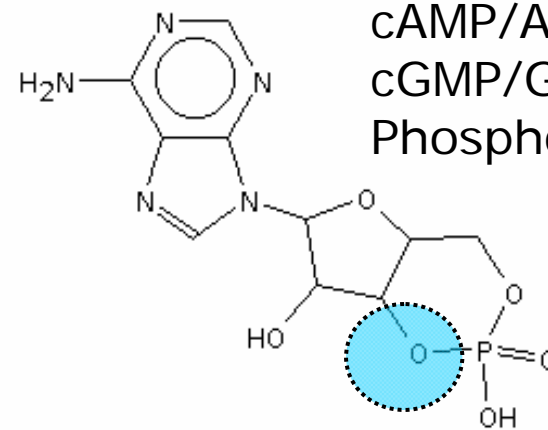
PAPS/PAP:
Sulfotransferases



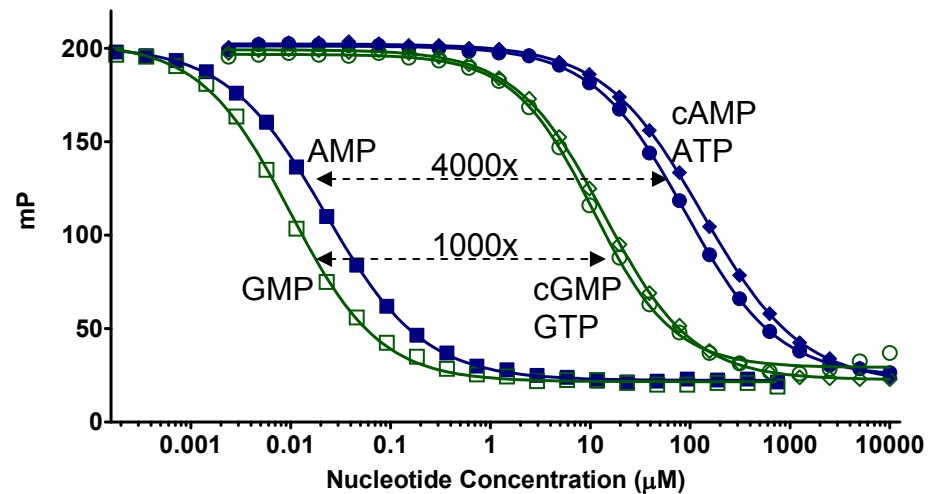
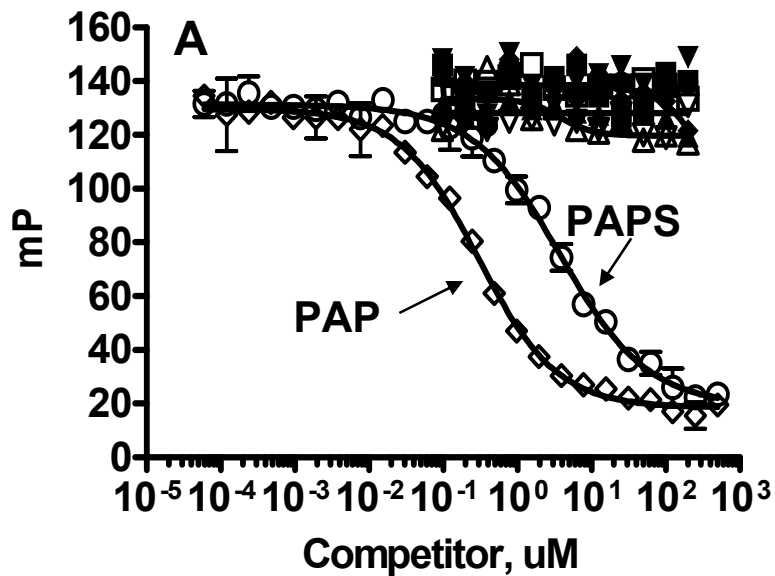
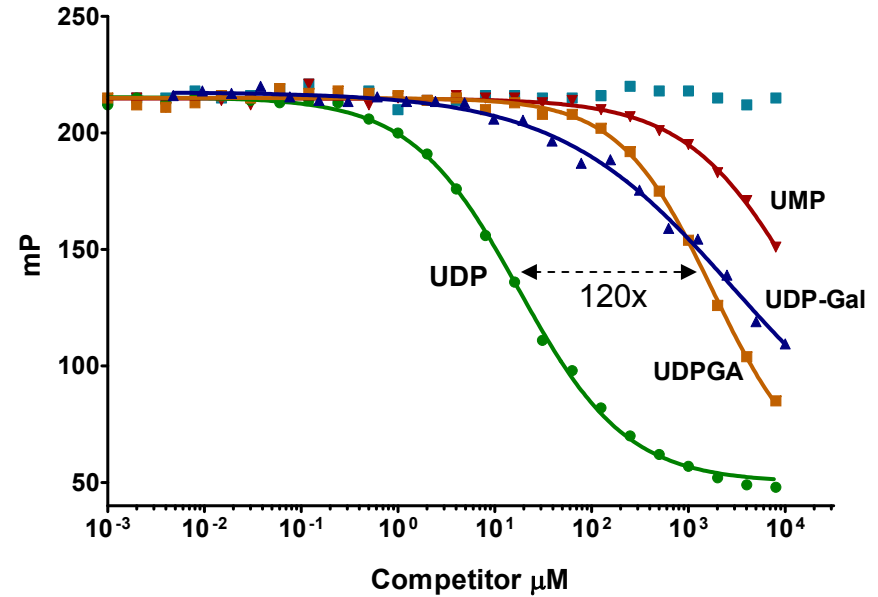
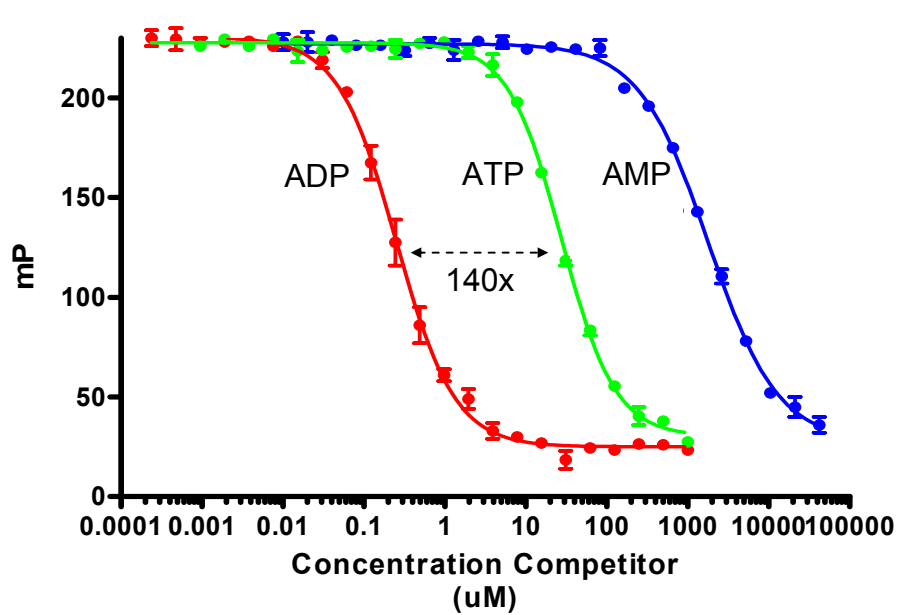
ATP/ADP: Kinases



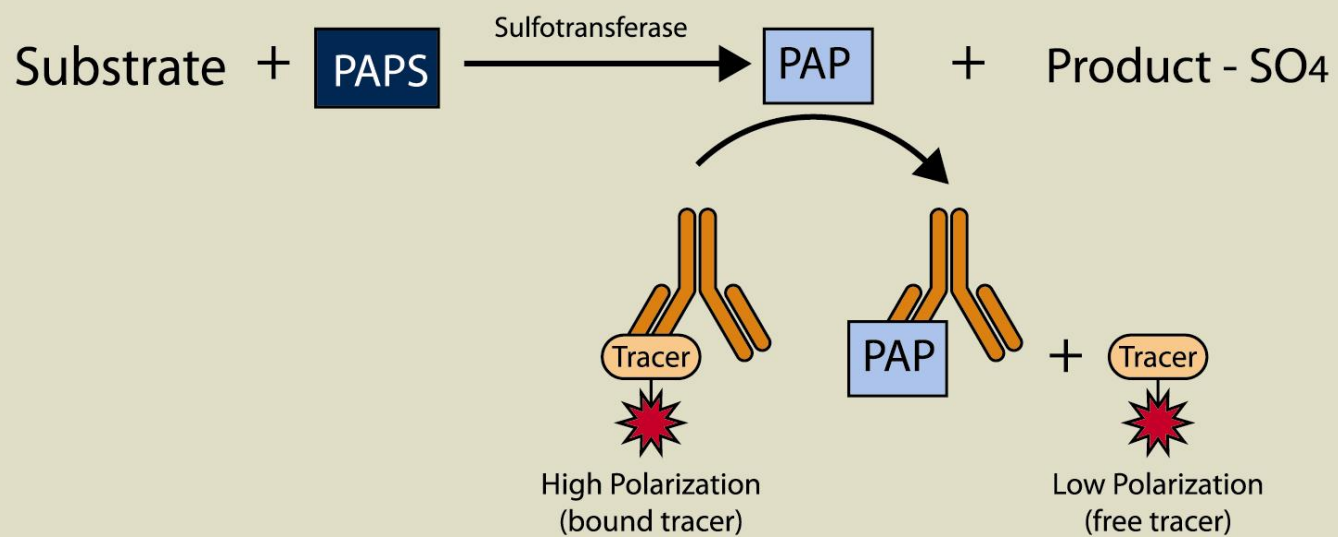
cAMP/AMP
cGMP/GMP:
Phosphodiesterases



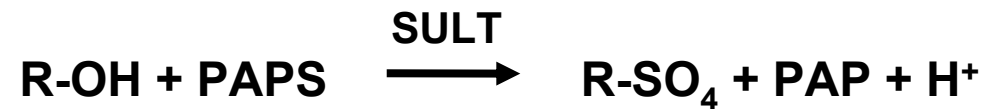
Selective Nucleotide Detection



Transcreener™ SULT Assay

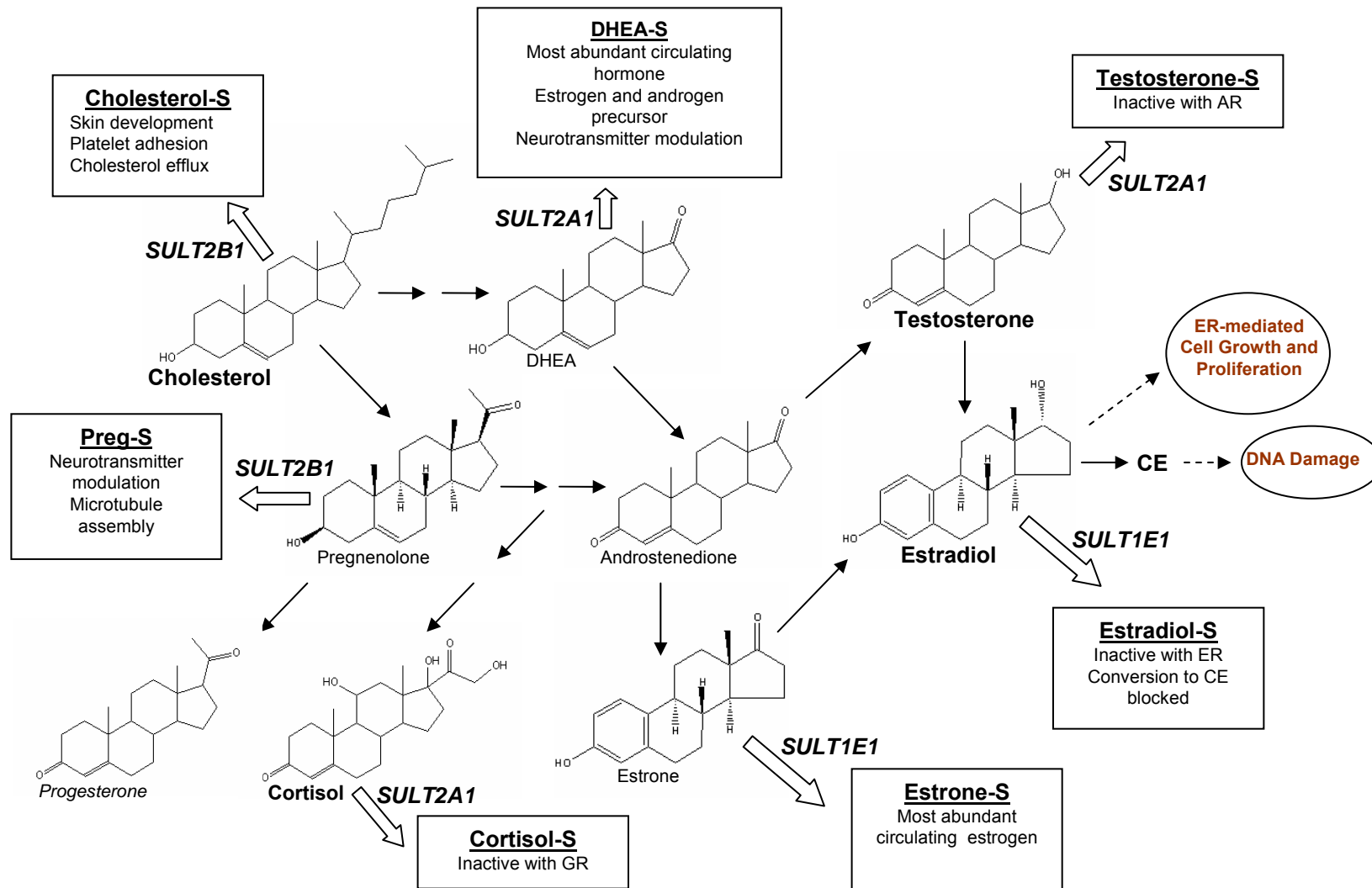


Sulfation: An Emerging Regulatory Modification

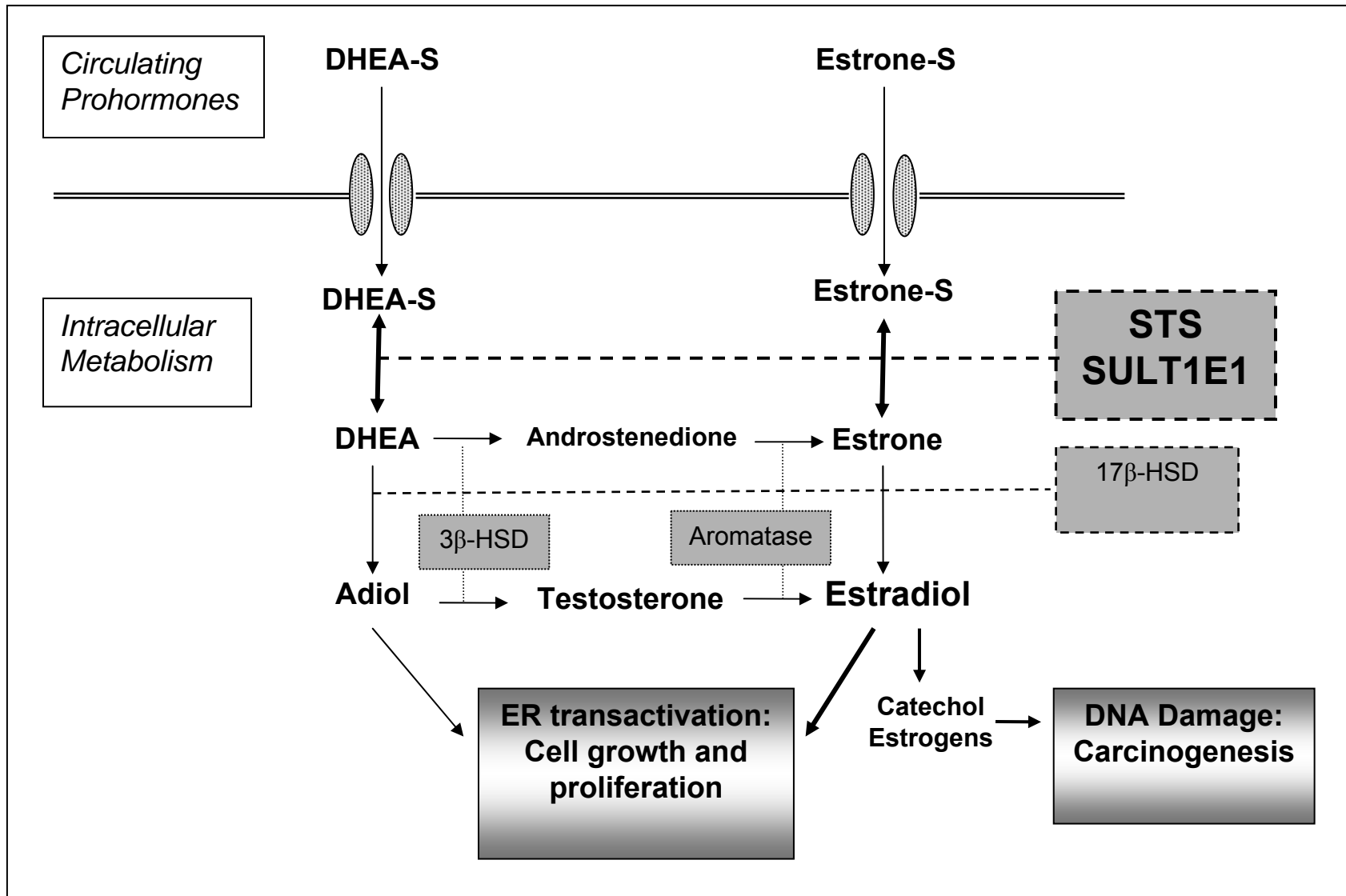


- >50 genes
- Targets include proteins, proteoglycans, xenobiotics, hormones, esp. steroids
- Reversible covalent regulatory cycles (sulfation/desulfation): dynamic regulation
- Therapeutic areas: drug metabolism, cancer, neurodegenerative disorders

The Joy of Sex Hormone Sulfation

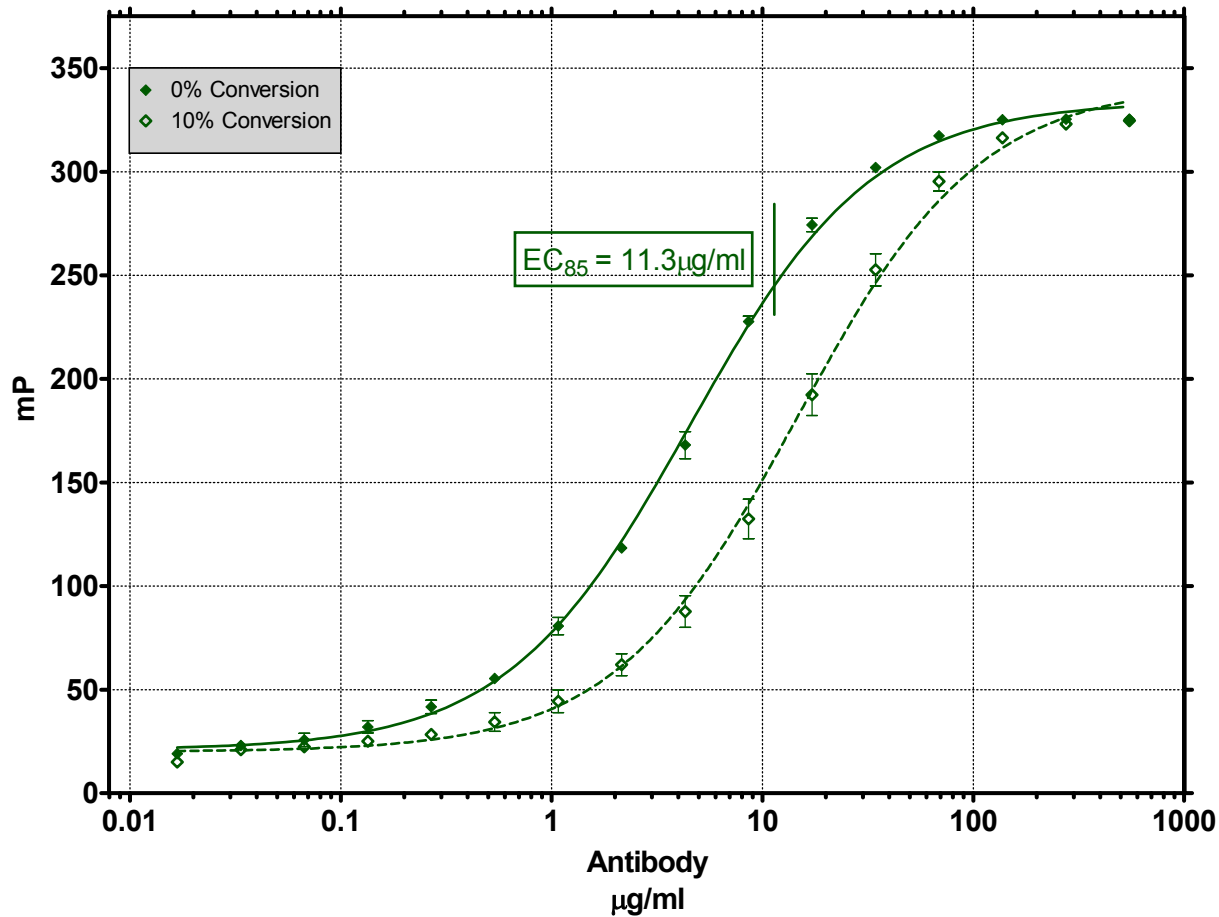


Intracrine Production of Estrogens in Mammary Epithelial Cells



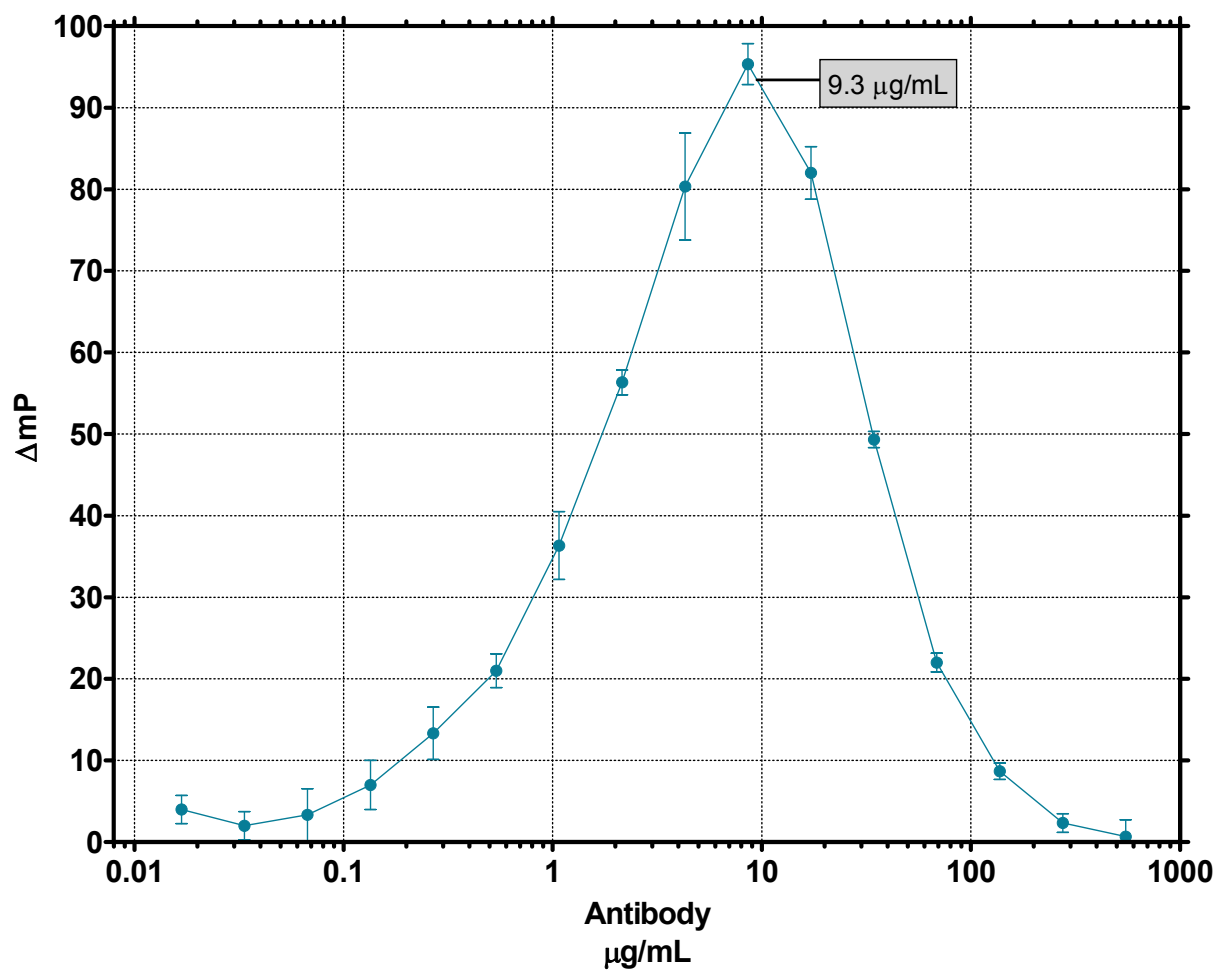
Optimization for Initial Velocity Determination:

1) Antibody Binding Curves @ 0 and 10% conversion of 15 μ M PAPS



Optimization for Initial Velocity Determination:

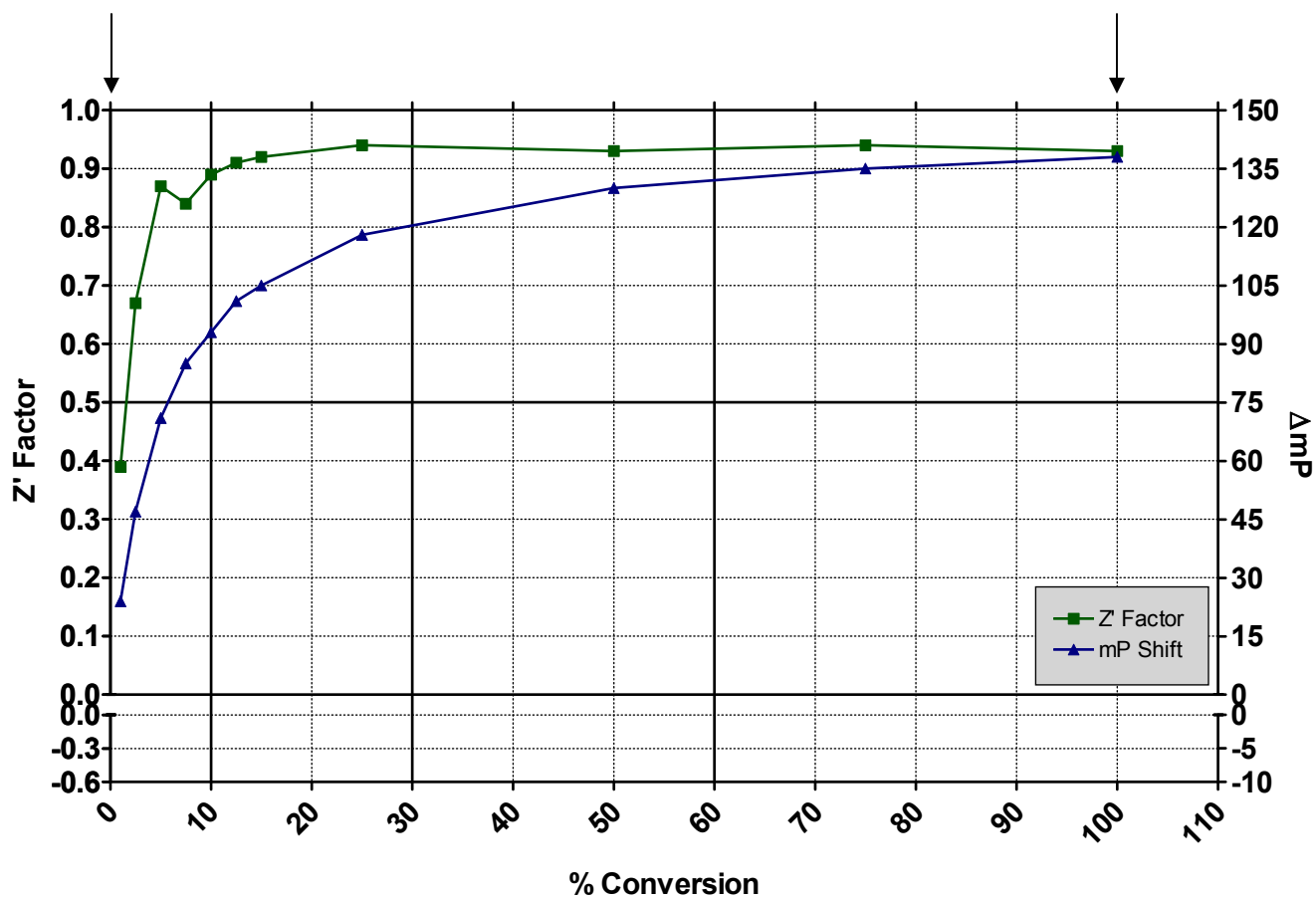
2) Determination of Optimal [Ab] for 10% conversion



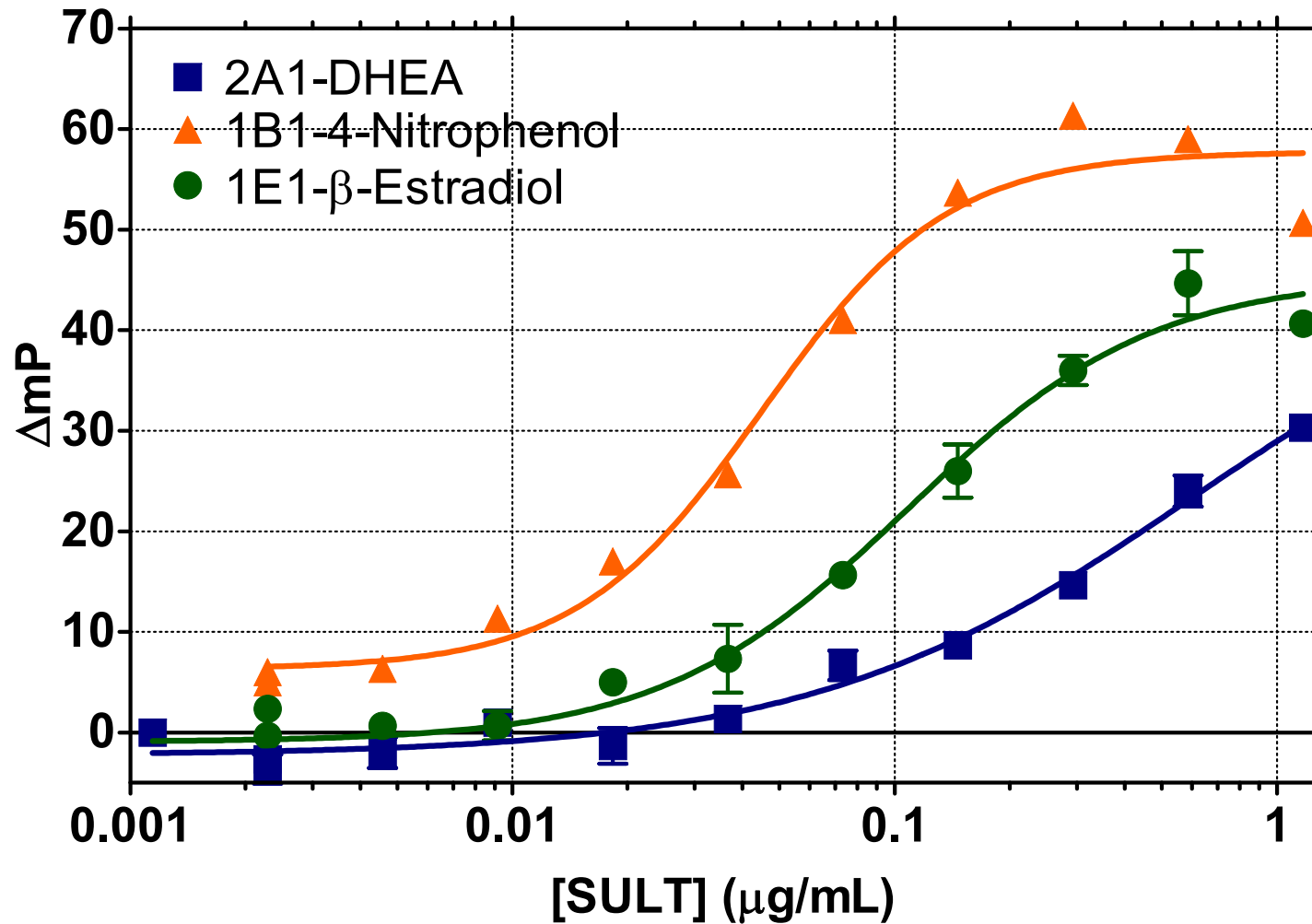
Mock Reaction Standard Curves

(15 μ M PAPS, 0 μ M PAP)

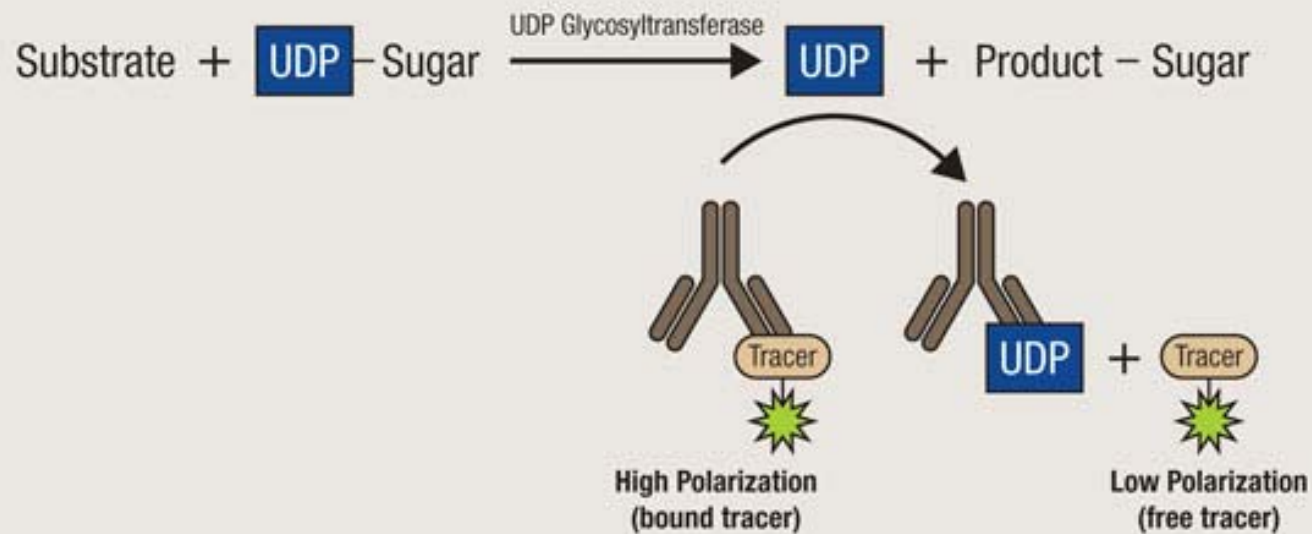
(0 μ M PAPS, 15 μ M PAP)



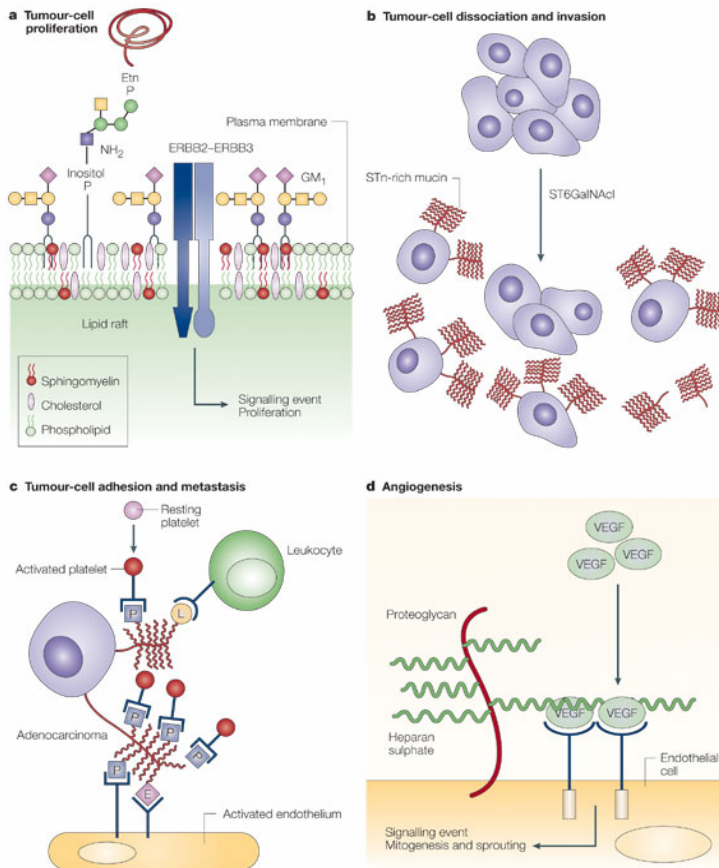
Detection of cytosolic SULT isoforms



Transcreener™ UGT-488P Assay



Glycosylation: An Extremely Diverse and Ubiquitous Regulatory Modification

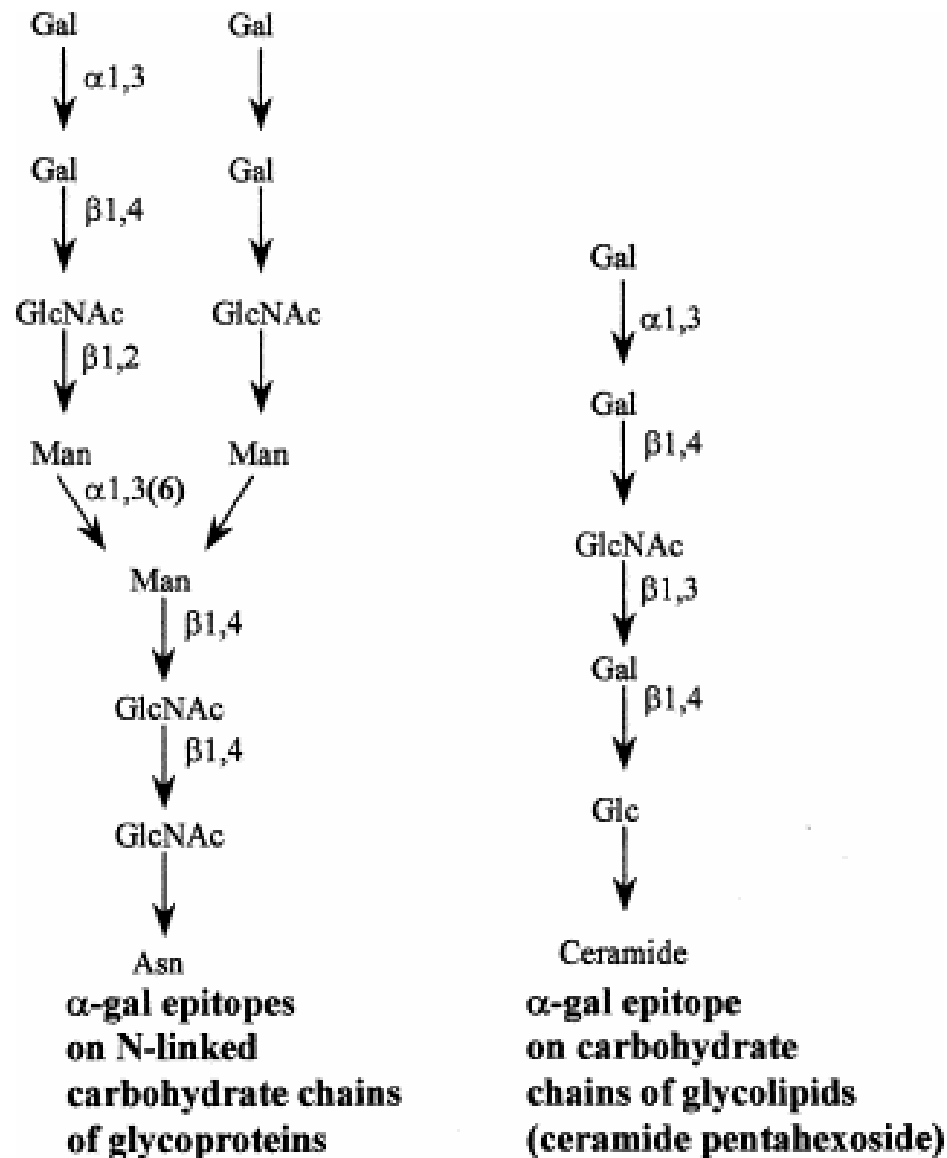


- Over 200 UGTs in humans
- Substrates: proteins, proteoglycans, lipids and small molecules
- Protein compartmentalization, xenobiotic metabolism, cell attachment
- Drug metabolism, cancer, inflammation, cystic fibrosis

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Nature Reviews | Cancer

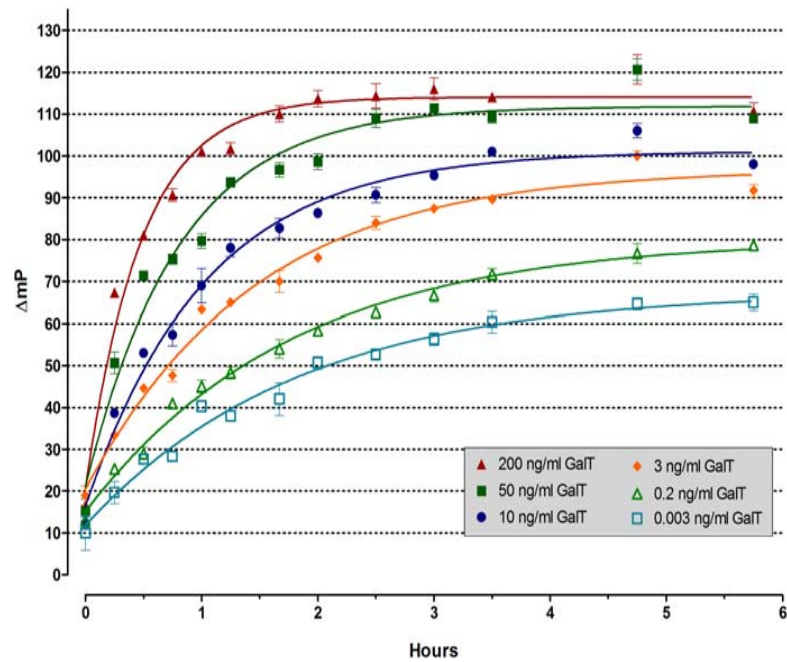
Fuster MM, Esko JD: The sweet and sour of cancer: glycans as novel therapeutic targets. *Nat Rev Cancer* (2005) 5(7): 526-42.

Transplant rejection: $\alpha 1,3$ Galactose

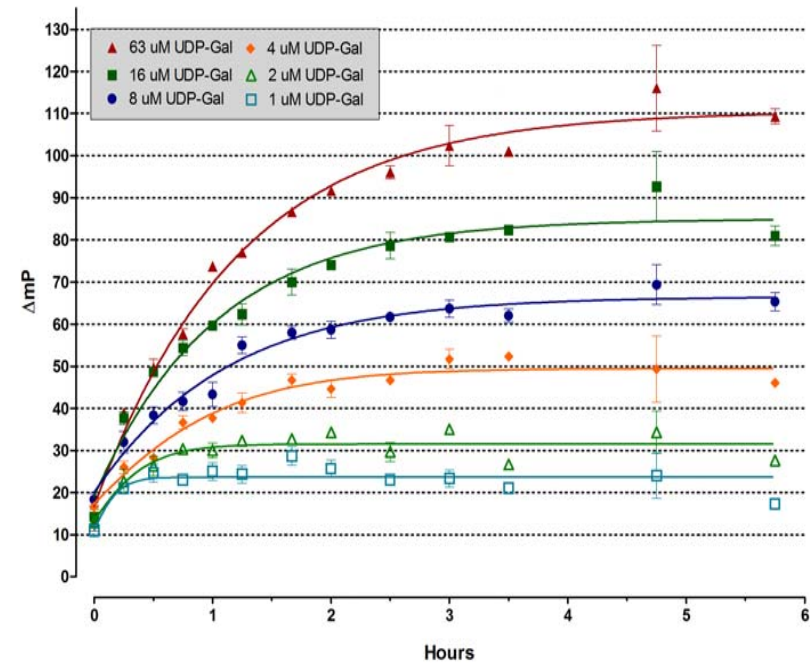


Time, Enzyme and Substrate Dependence

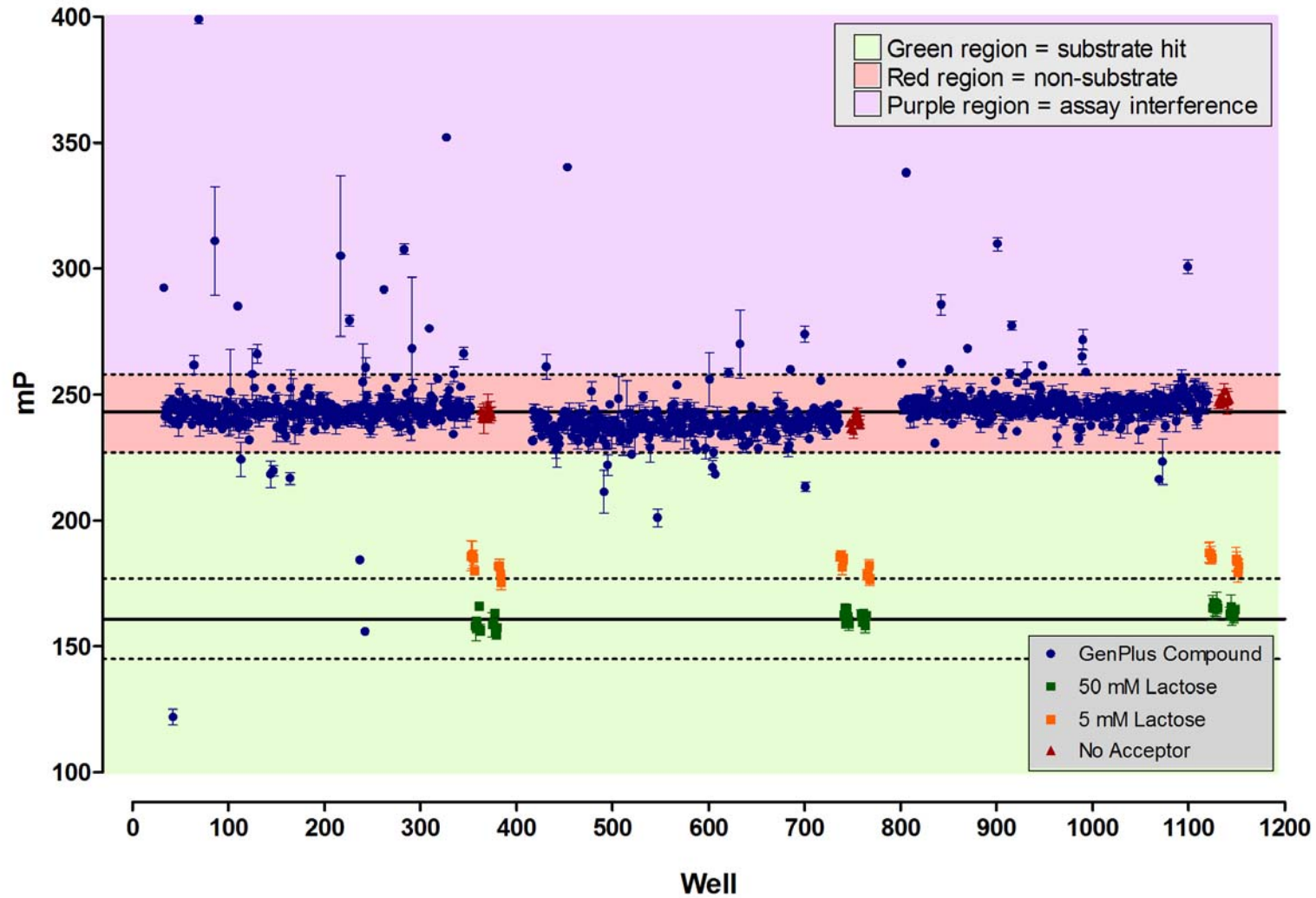
α -1,3-Galactosyltransferase Titration



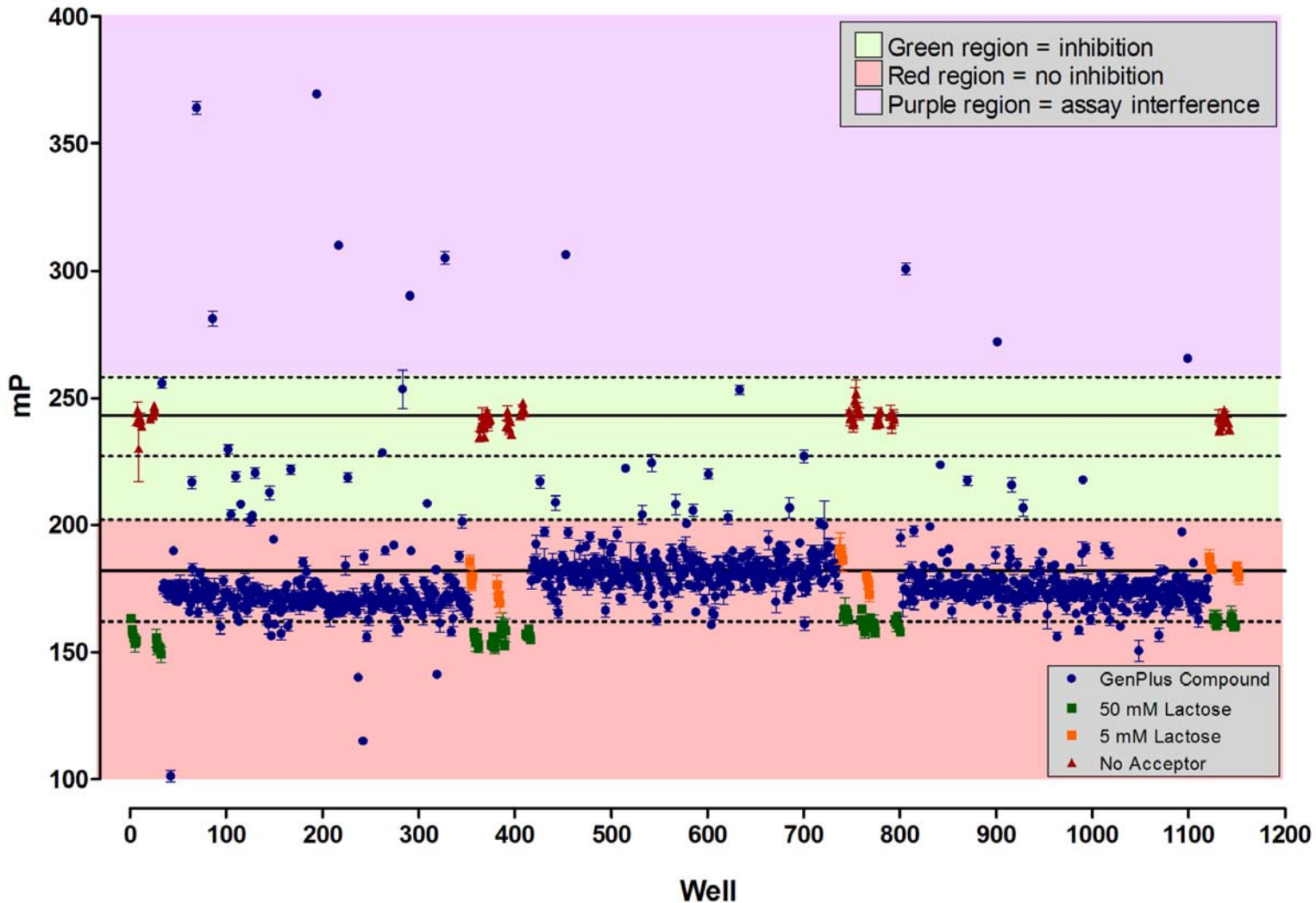
UDP-Galactose Titration



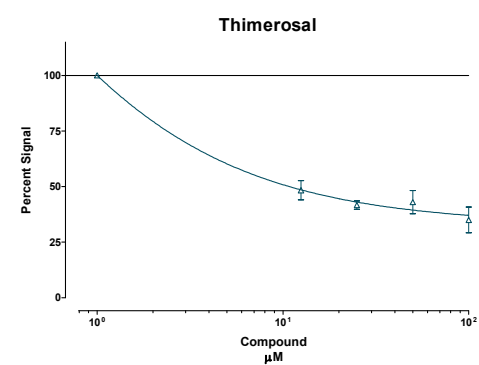
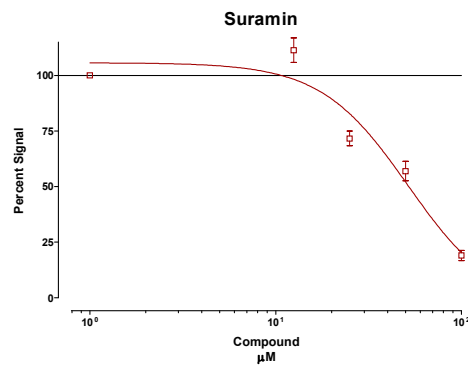
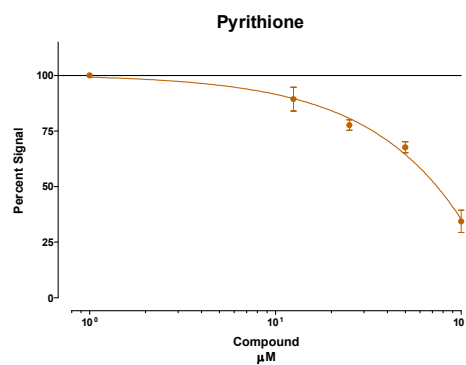
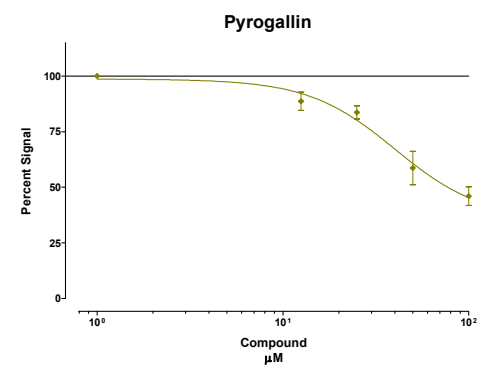
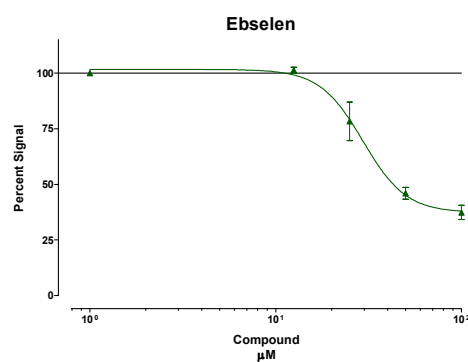
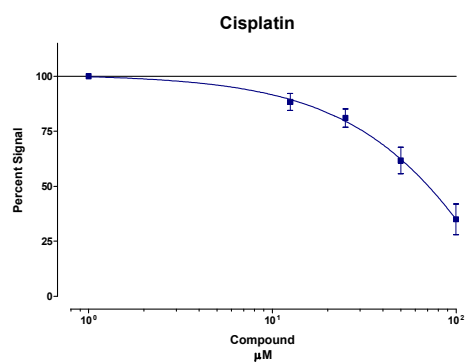
Transcreener™ UGT: Screening for Acceptors



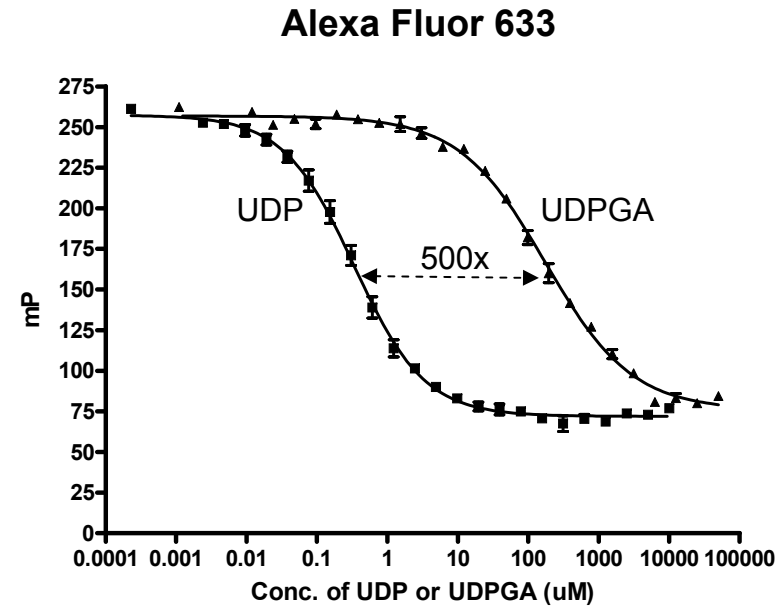
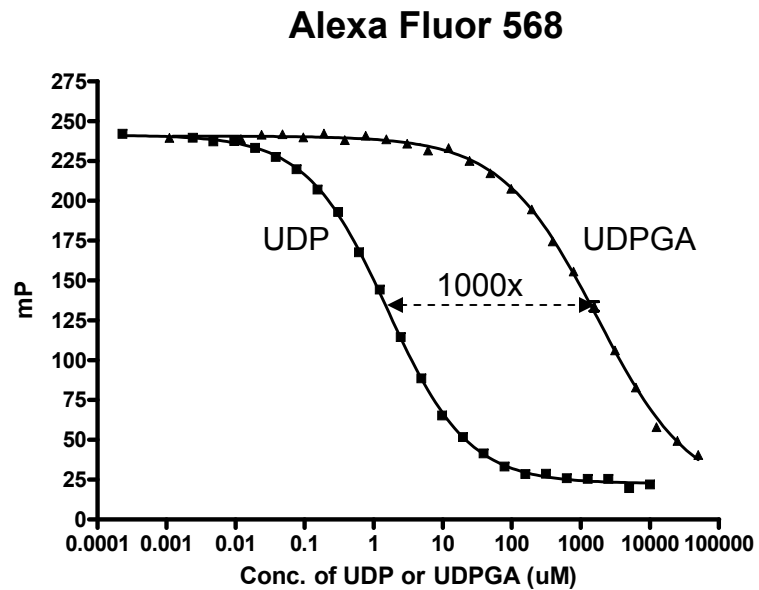
Transcreener™ UGT: UDP-Galactosyltransferase Inhibitor Screen



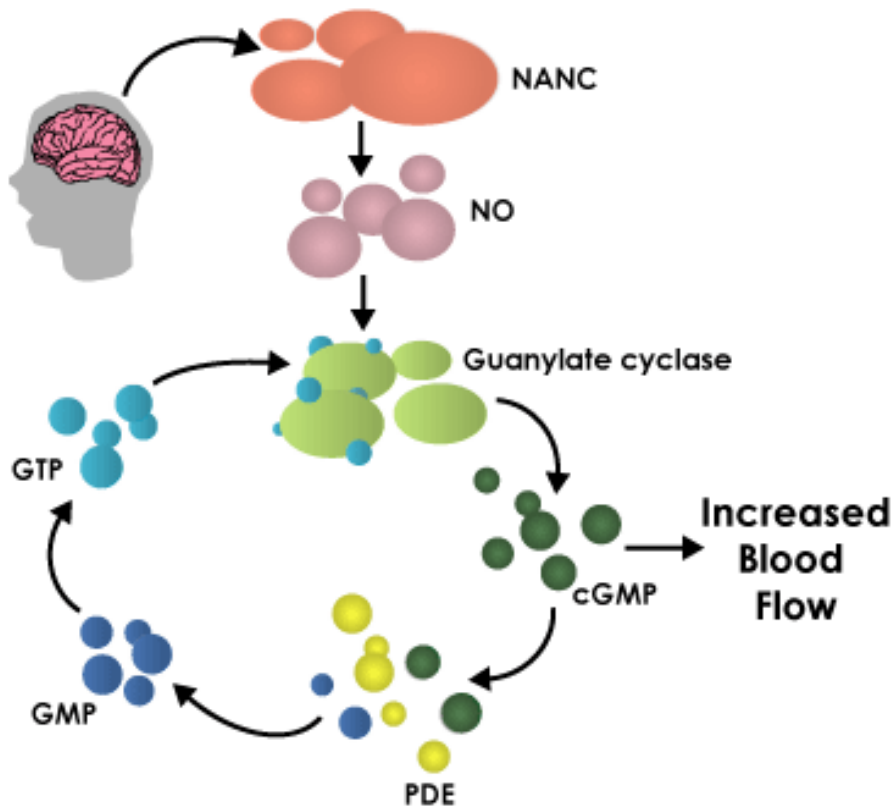
UDP-Galactosyltransferase Inhibitors: Dose Response



Transcreener UGT: Tracer Effects on Selectivity

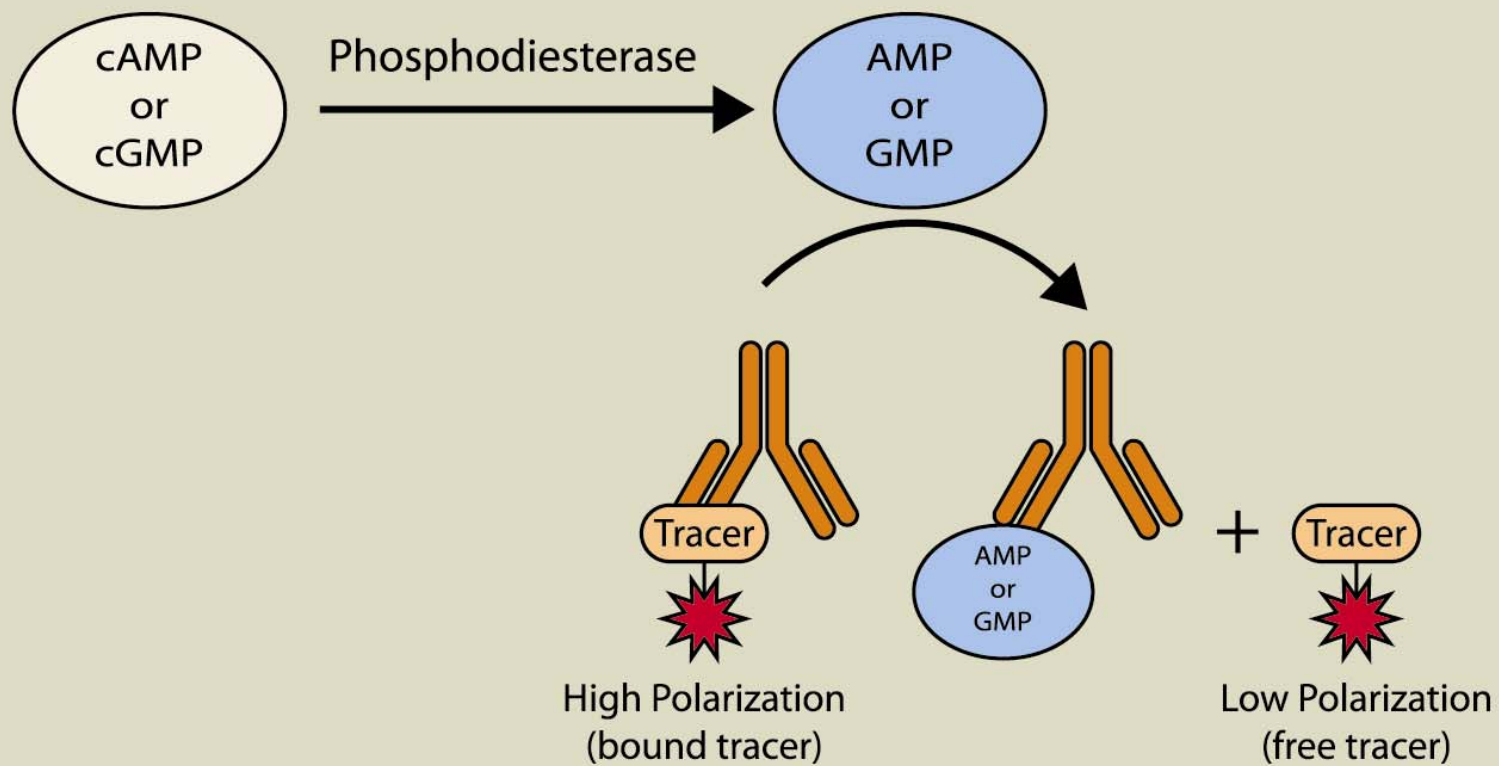


Cyclic Nucleotide Phosphodiesterases

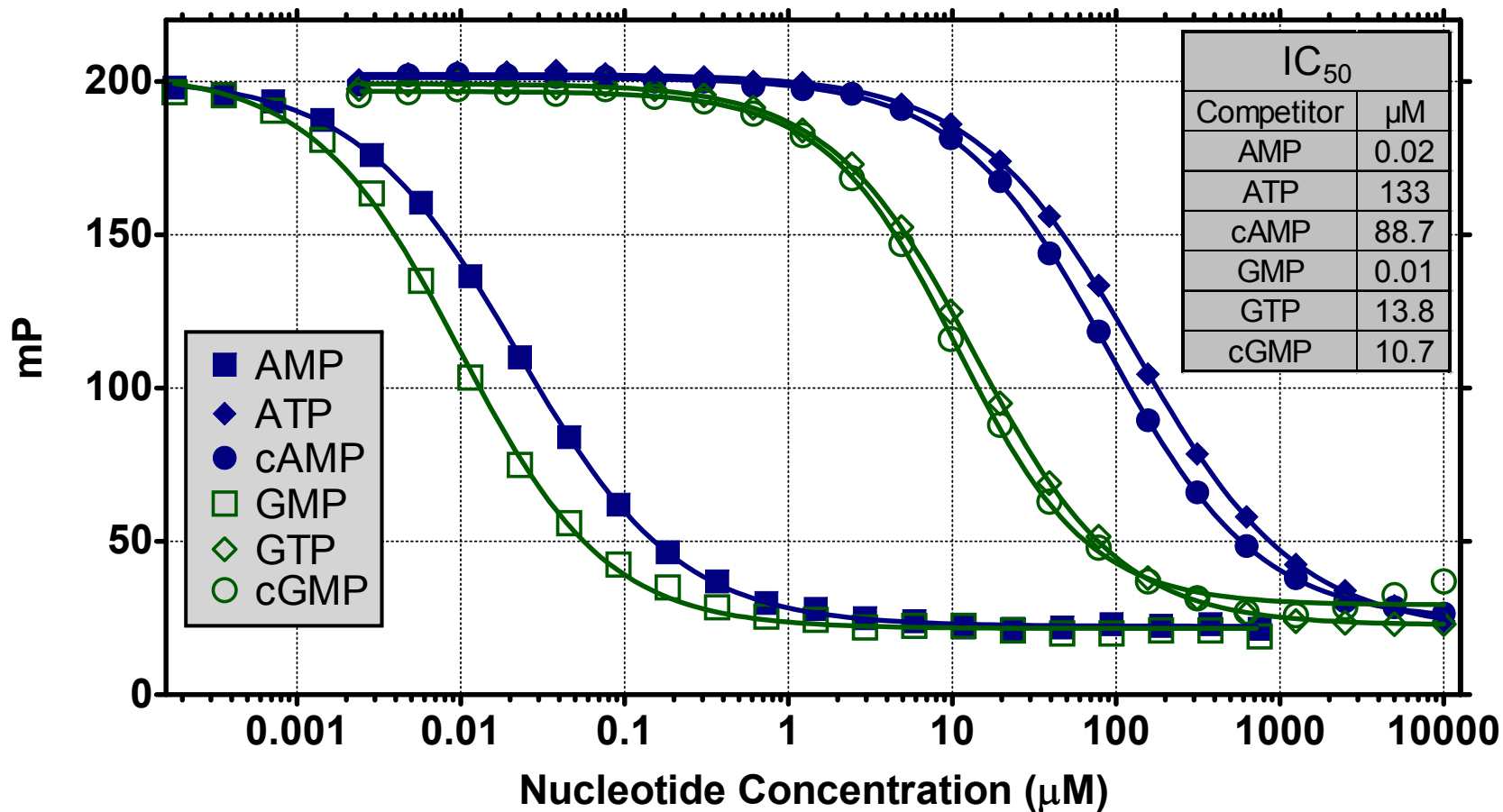


- cAMP \rightarrow AMP
- cGMP \rightarrow GMP
- 11 PDE families
- 21 genes
- At least 80 splice variants
- PDE 4 – inflammation, CNS
- PDE 5 – sexual dysfunction, hypertension, CNS

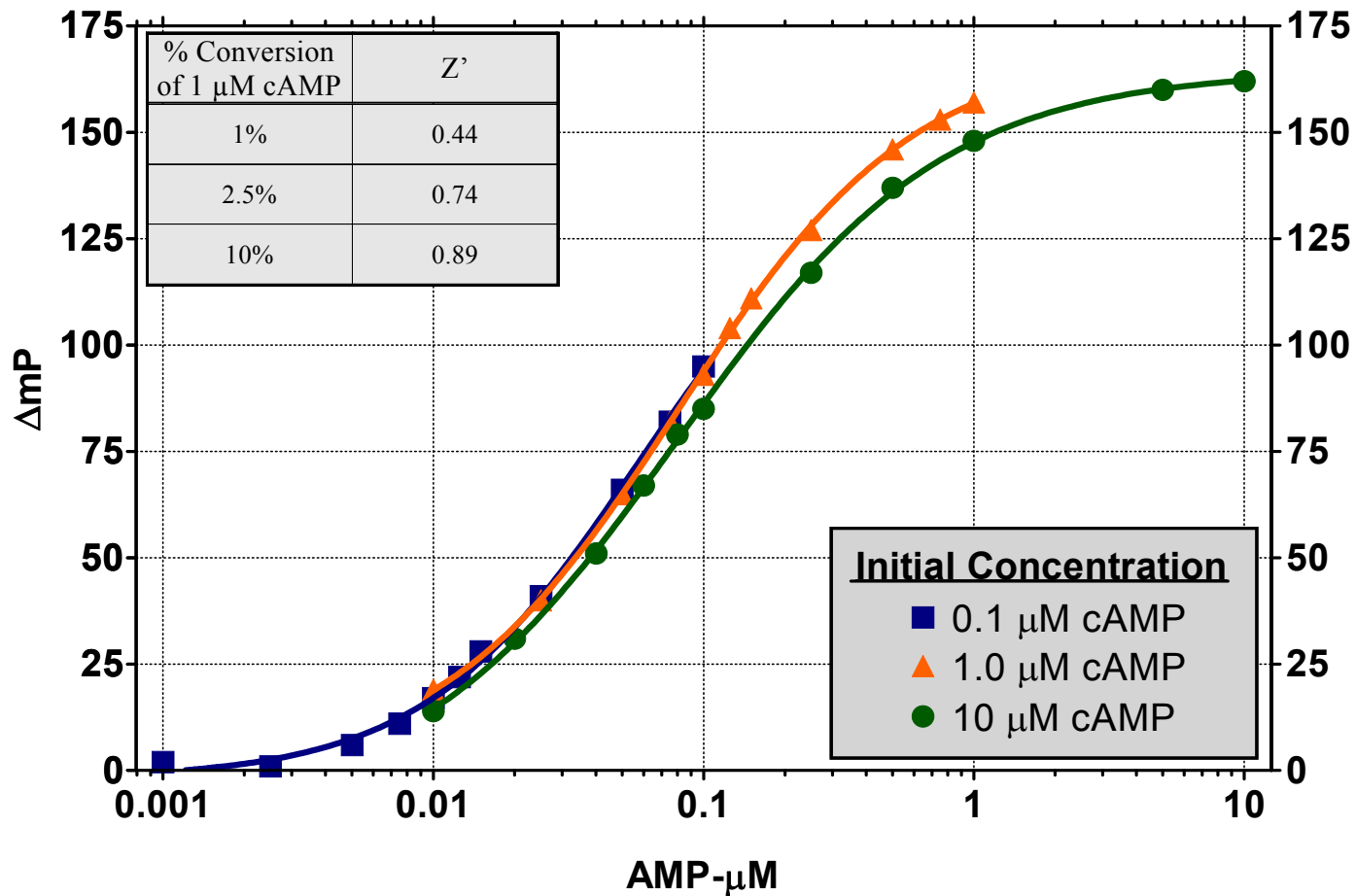
Transcreener™ PDE Assay



Transcreener™ PDE: Ab/Tracer Selectivity Profile

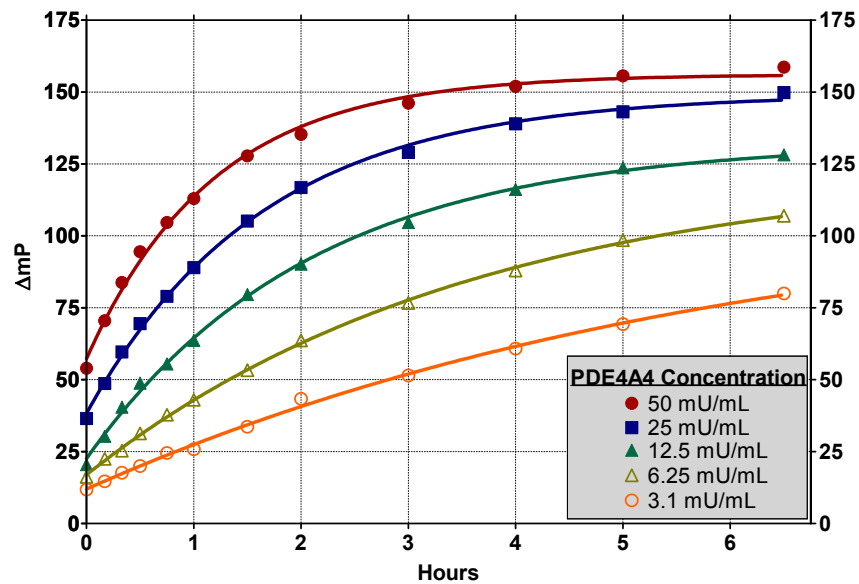


Transcreener™ PDE: Mock Rxn Standard Curves (cAMP → AMP)

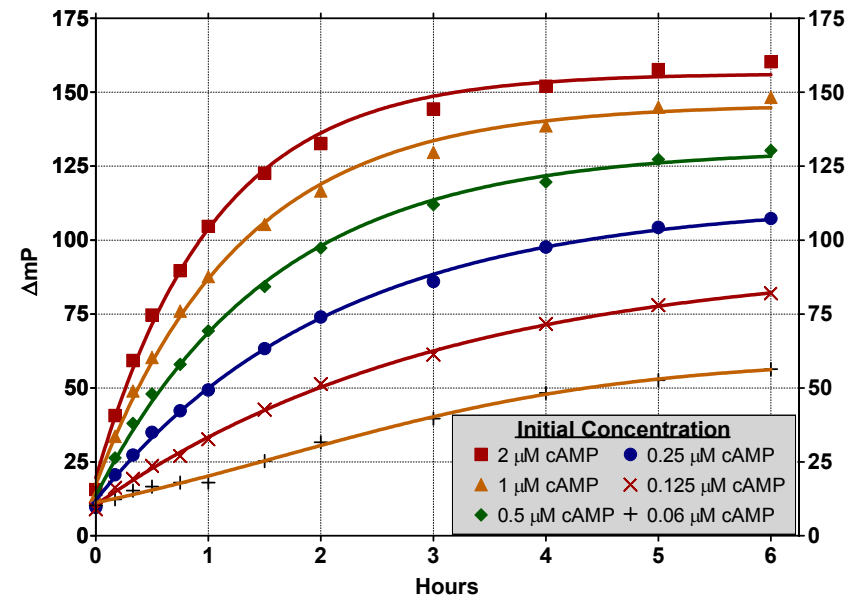


Transcreener™ PDE: Time, Enzyme and Substrate Dependence

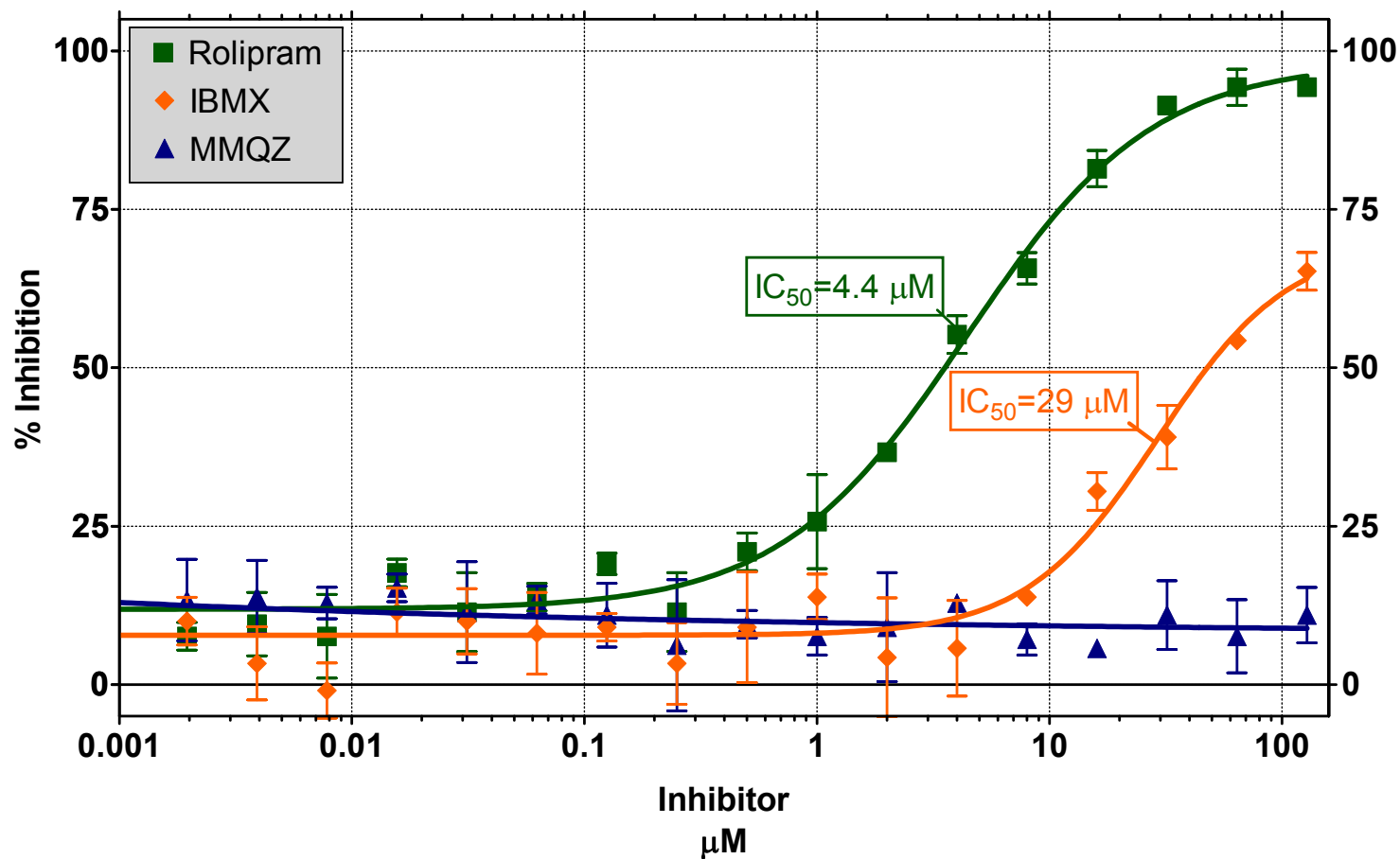
PDE4A4 Dependency with cAMP



cAMP Dependency with PDE4A4



PDE 4A4 Inhibition



Transcreener™ PDE Assay

AMP Producing Enzymes	Examples
Ligases (ATP → AMP + P_{Pi})	
Nucleic acid ligases	RNA, DNA ligases
Aminoacyl-tRNA synthetases	Glycine-tRNA ligase
Protein ligases	Ubiquitin-protein ligase
Acyl-CoA synthetases	Long-chain-fatty-acid-CoA ligase
Phosphodiesterases	
Cyclic nucleotide phosphodiesterases	PDE 4 (cAMP), PDE 5 (cGMP)
5'-nucleotide phosphodiesterases	PDE I

Transcreener™ Kinase Assay



ADP Producing Enzymes	Examples
Kinases	
Protein kinases	PKA (Ser/Thr), Abl1 (Tyr)
Lipid kinases	Phosphatidylinositol-3-Kinase
Carbohydrate kinases	Phosphofructo-kinase-1
Nucleic acid kinases	Polynucleotide kinase
ATPases	
Chaperonins	HSP 90
Drug Transporters	MDR1
Ion Channels	Na ⁺ /K ⁺ ATPase
Molecular Motors	Kinesin Eg5
Nucleic Acid Enzymes	
DNA unwinding	Helicases, gyrases, topoisomerases
DNA Recombination	RecA
DNA Repair	MutH/S/L endonuclease
Carboxyltransferases	Acetyl CoA carboxylase

Summary

- **Transcreener™ Platform:** Homogenous, fluorescent immunodetection of nucleotides with high selectivity for products vs. substrates
- **PAP/PAPS:** SULTs
- **UDP/UDPGA:** UGTs
- **AMP(GMP)/cAMP(cGMP):** PDEs, Ligases, Synthetases
- **ADP/ATP:** Kinases, ATPases, Nucleic Acid Enzymes, Carboxyltransferases



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SCREENING SIMPLIFIED. DRUG DISCOVERY MULTIPLIED.