Interrogating Diverse Target Families by Homogenous Immunodetection of Nucleotides

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Group transferases catalyze the transfer of a chemical group from a donor to an acceptor substrate. These enzyme families are rich in therapeutic targets because they are involved in tunable, covalent regulatory cycles. Their roles in controlling macromolecule function are well understood, e.g., phosphorylation of proteins, methylation of proteins and DNA, and histone acetylation, and their roles in endogenous small molecule regulation are just beginning to be understood – e.g. sulfation of steroid hormones. The products of a group transferase reaction are the specific conjugated acceptor and the invariant 'donor product', e.g. ADP for kinases. Historically, transferase assays are performed by detecting the highly variable, conjugated acceptor and involve separation steps or coupled enzymatic reactions.

In this study, we demonstrate how the Transcreener Assay platform enables homogenous immunodetection of invariant nucleotide donor products, enabling robust, generic detection of entire families of drug targets. Among the enzyme classes presented will be kinases and ATPases, glycosylatransferases, sulfotransferases, and phosphodiesterases. Only one assay for each donor product is required to screen an entire enzyme family without the need for coupled enzymatic reactions.

Figure 1.

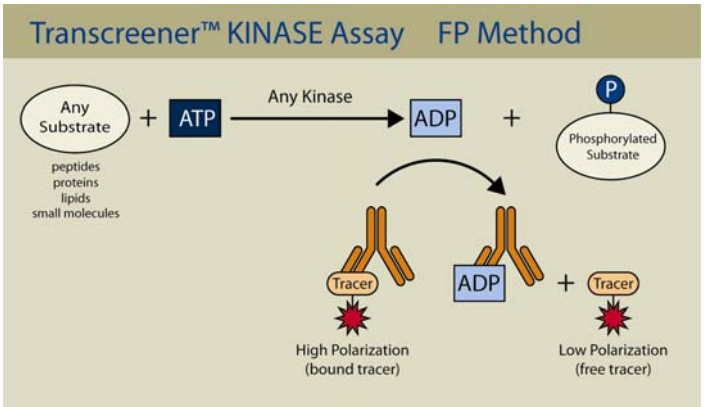
Group Transfer Enzymes Control Diverse Biomolecules and Disease Pathways

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

Group transferases are enzymes that catalyze the transfer of a chemical group from a donor to an acceptor substrate. These families are rich in targets because they are involved in tunable covalent regulatory cycles. Their role in controlling macromolecule function is well understood: e.g. kinases with proteins, methyltransferases with proteins and DNA, histone acetylation, but their role in endogenous small molecule regulation, such as sulfation of steroid hormones, is just beginning to be understood.

Figure 2.

Transcreener™ Assay Platform: Imunodetection of Invariant Reaction Products



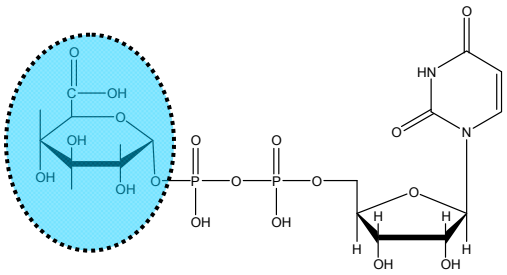
The Transcreener Assay Platform is based on homogenous detection of invariant nucleotide products, which enables a single set of reagents to be used across an entire family of group transfer enzyme. The Transcreener Kinase Assay, which relies on ADP detection, is shown here. Transcreener assays can be formatted for many different detection modes; all of the BellBrook Transcreener Assays have been formatted for fluorescence polarization, as shown here. When the Ab is bound to the tracer, the polarization value is high and when the tracer is displaced by the product of the enzyme reaction, the polarization value is low. The Transcreener Kinase Assay is also avaliable in a TR-FRET format.

Figure 3.

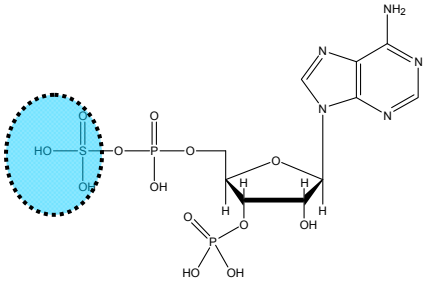
Transcreener™ Platform: Selective Nucleotide Detection

The Transcreener Platform relies on antibodies that recognize reaction products (e.g., ADP) with a high degree of selectivity over substrates (e.g., ATP) that differ by as little as a single phosphate group.

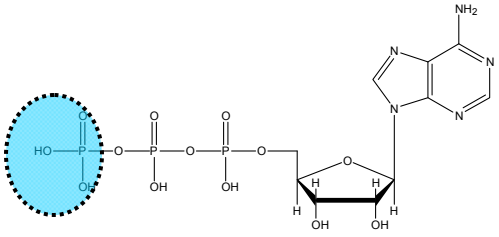
UDPGA/UDP: Glycosyltransferases



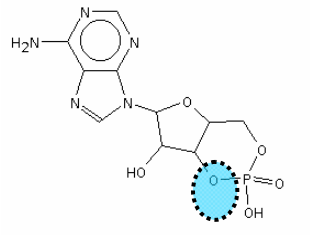
PAPS/PAP: Sulfotransferases



ATP/ADP: Kinases cAMP/AMP



cGMP/GMP:Phosphidesterases



Transcreener reagents, antibodies and tracers, have been developed for detection of ADP, UDP, PAP, and AMP/GMP for screening respectively Kinases, Glycosyltransferases, Sulfotransferases and Phosphodiesterases. Note that there are applications oustide these primary target familes as well: e.g., ATPases and Carboxyltransferases (ADP), Ligases (AMP).

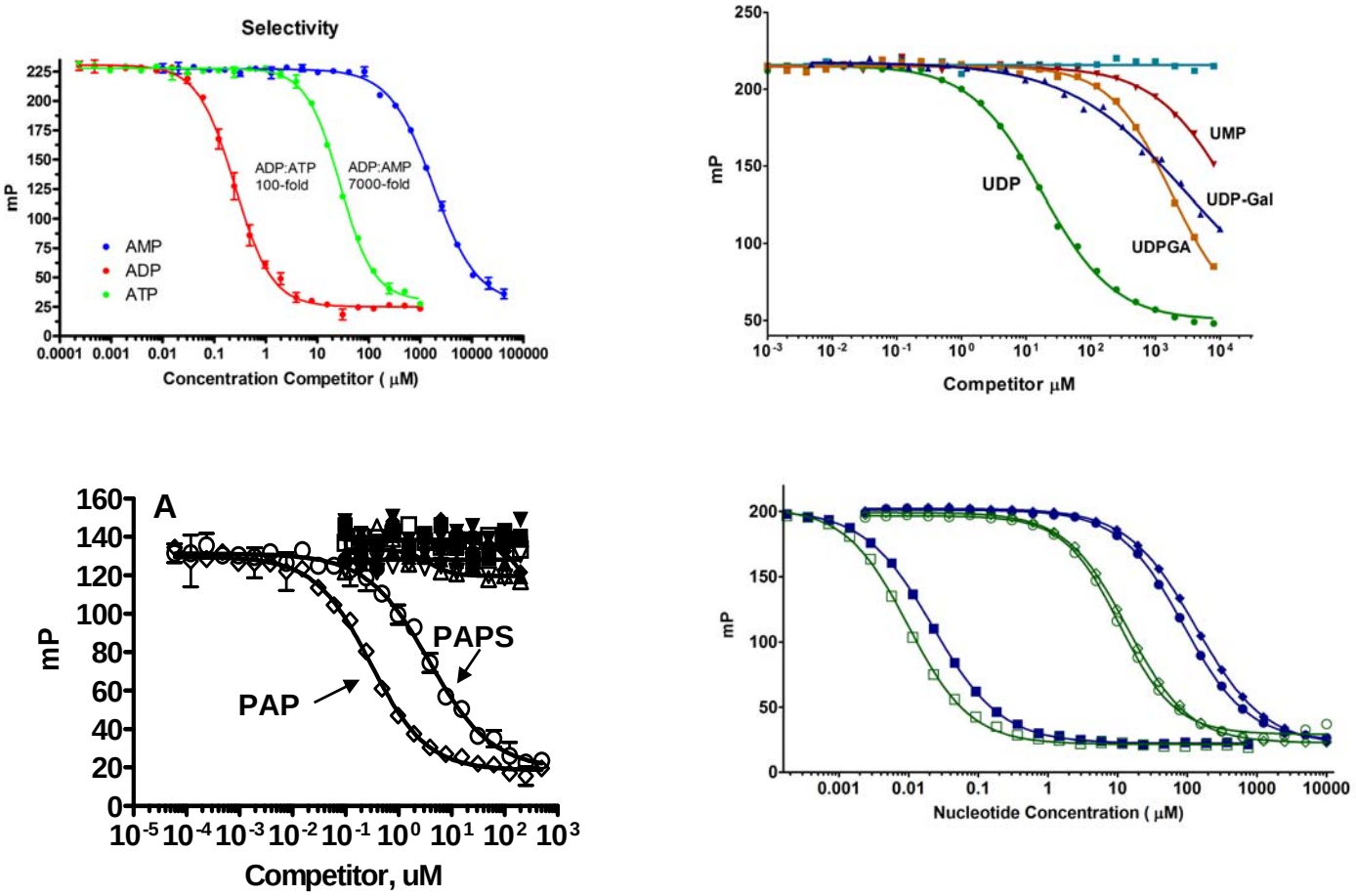
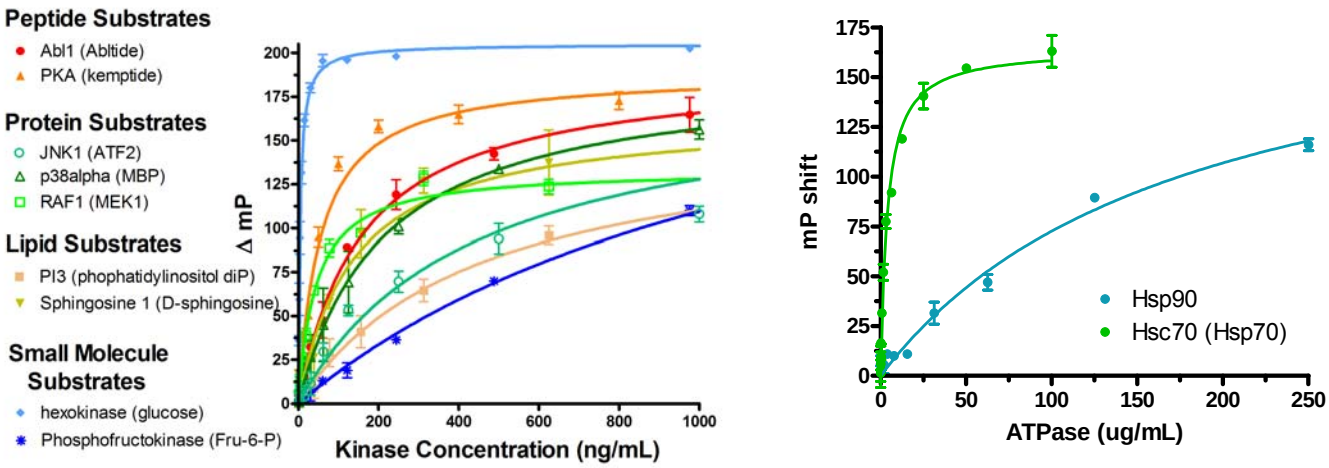


Figure 4.

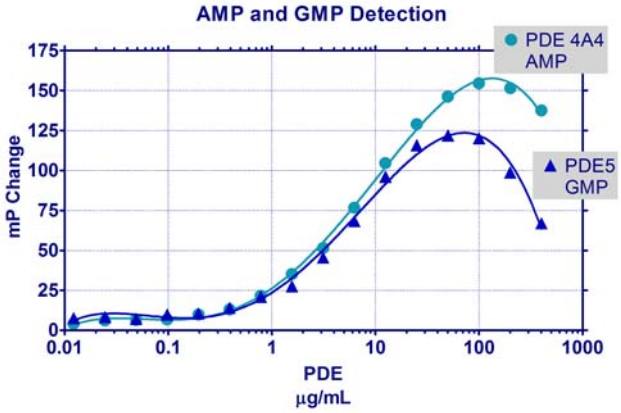
Transcreener™ Kinase Assay Allows Screening of Protein, Lipid and Carbohydrate Kinases and ATPases



Detection of diverse members of the kinase superfamily with the Transcreener™ KINASE Assay. Indicated amounts of kinases or ATPases were incubated in 384 well plates with their corresponding acceptor substrates and ATP at or near its Km value. The Trancreener™ detection reagents – monoclonal antibody and tracer – were added with metal chelator to stop the reactions, and the fluorescence polarization values were read on a Tecan Ultra.

Figure 5.

Transcreener™ PDE Assay Detects AMP- and GMP- Producing Enzymes

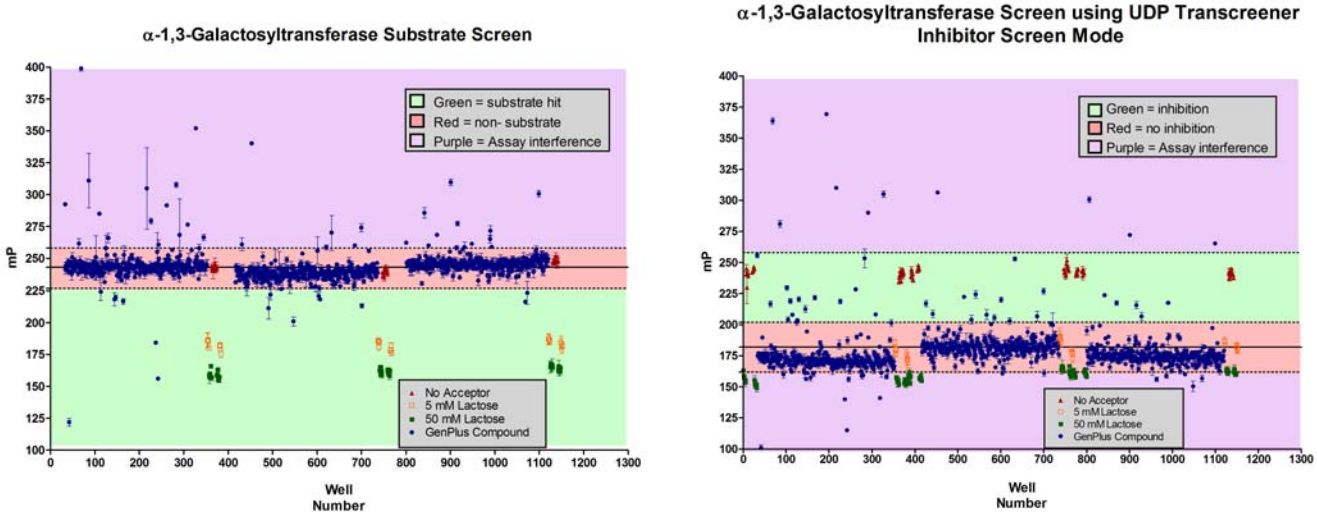


Enzyme Dependence for PDE4A4 and PDE5. Enzyme dependent signal was demonstrated with PDE4A4 and PDE5 using their respective substrates cAMP and cGMP with one set of detection reagents. cAMP for PDE4A4 and cGMP for PDE5 were both present at 1 µM. Reactions were incubated at 30°C.

ΔmP = mP (no substrate) – mP (containing substrate)

Figure 6.

Transcreener™ UGT: Screening for Small Molecule Acceptors and Inhibitor for a UDP-Galactosyltransferase



The Transcreener™ Assay Platform is unique in that it provides the means to screen for both substrates and inhibitors. The enzyme used in this study was a His-tagged, recombinant alpha-1,3-GalT expressed in and purified from E. coli. Of the compounds in this library: 1.7% were classified as substrate hits, 94.4% demonstrated no activity, and 2.6% interfered with assay signal. GenPlus compounds were used at 10 µM.

The same compound set used in the substrate screen was used in the inhibitor screen. Compounds were tested for their ability to inhibit galactose conjugation of the acceptor substrate lactose.

Conclusions:

- Group transferases enzyme families are rich in drug targets because of their key role in tunable covalent regulatory cycles.
- Development of highly selective antibodies and fluorescent nucleotide tracers enables homogenous detection of nucleotide enzyme products in the presence of structurally similar substrates.
- The Transcreener™ Kinase Assay (ADP detection) can be used to detect any ATP-dependent phosphotransferase reaction, regardless of the acceptor substrate, including ATPases (H20 acceptor).
- The Transcreener™ PDE assay enables detection of cAMP and cGMP-dependent phosphodiesterases using a single set of reagents.
- The Transcreener™ UGT Assay (UDP detection) allows screening for glycosyltransferase acceptors and inhibitors.

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