Screening Small Molecule Regulatory Enzymes: Glycosyltransferases, Sulfotransferases and Phosphotransferases

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Introduction

Group transferases catalyze reactions of the general form: Donor-X + Acceptor → Donor Product + Acceptor-X. In many cases, the adduct is activated by a high energy bond to a nucleotide or a nucleotide-containing cofactor; e.g. ATP for phosphorylation. 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 and methylation of proteins and DNA; however their roles in endogenous small molecule regulation are just beginning to be understood. Similar to phosphorylation of proteins, covalent modification is used to regulate the stability and biological activity of endogenous hormones and metabolites. This regulatory function is intertwined with their role in the conjugation of drugs and xenobiotics. Thus the development of improved methods for screening small molecule conjugating enzymes is important both to explore their potential as therapeutic targets and to reduce the potential for small molecule drug-drug and drug-hormone interactions. In this study, we demonstrate how the Transcreener Assay platform - homogenous immunodetection of nucleotides - enables robust, generic detection of group transferases with small molecule acceptor substrates, including glycosyltransferases, sulfotransferases, and phosphotransferases.

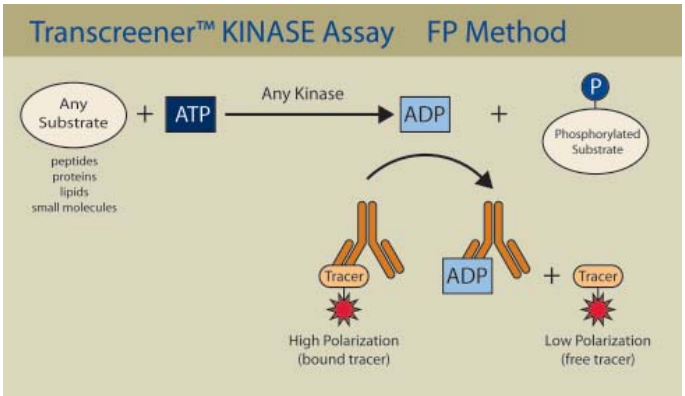
Acceptor Substrates for Key Group Transferase Enzyme Families

Target Family	Substrates (small molecules are in red)	Disease Areas
Kinases (>650)	Proteins, Nucleic Acids, Nucleotides, Lipids, Carbohydrates, Water, (ATPase)	Cancer, Inflammation, Diabetes, Cardiovascular, Neurological
Glyscosyltransferases (>200)	Proteins, proteoglycans, Drugs/xenobiotics, Steroid hormones, Lipids, Bile Acids, Retinoids, Bilirubin	Drug Metabolism, Cancer, Antimicrobials
Sulfotransferases (>50)	Proteins, proteoglycans, Drugs/xenobiotics, Steroid hormones, Catecholamines, Cholesterol	Drug Metabolism, Cancer, Cardiovascular, Neurological
Methyltransferases (>50)	Proteins, DNA, Drugs/xenobiotics, Catecholamines, Lipids, Histamine, Nicotinamide	Drug Metabolism, Cancer, Neurological

Covalent modification of hormones regulates their activity similarly to the regulation of protein activity by phosphorylation. It can be used to increase or decrease the stability of a hormone or to change its activity at a receptor protein. Catechol Methyltransferase (COMT) is an example of a small molecule group transferase that has been targeted therapeutically. Drugs that inhibit COMT are used to increase the duration of L-DOPA effects in the treatment Parkinson's disease and thus reduce the time patients spend in the relatively immobile "off" phase.

Figure 1.

Transcreener™ Assay Platform: Homogenous Immunodetection of Invariant Reaction Products

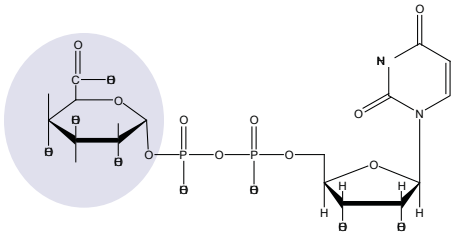


The Transcreener Assay Platform is based on homogenous detection of invariant nucleotide products, which enables a single set of reagents to be used across an entire family of group transfer enzyme. Transcreener assays can be formatted for many different detection modes; all of the BellBrook Transcreener Assays have been formatted for fluorescence polarization, as shown here. When the antibody 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.

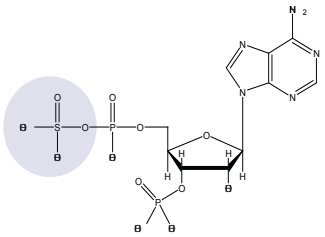
Figure 2.

Transcreener™ Platform: Selective Nucleotide Detection

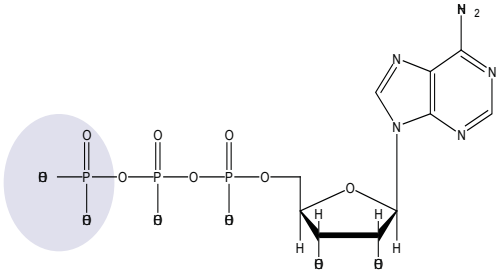
UDPGA/UDP: Glycosyltransferases



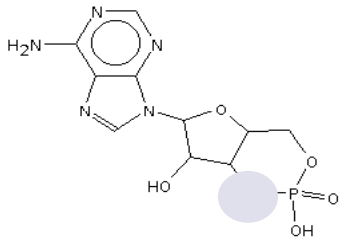
PAPS/PAP: Sulfotransferases



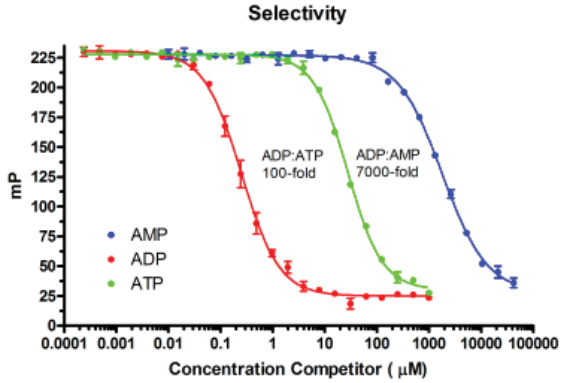
ATP/ADP: Kinases

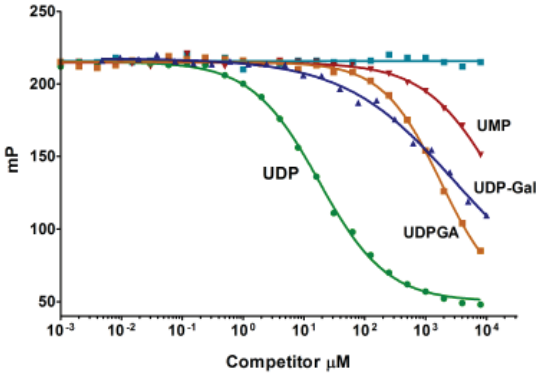


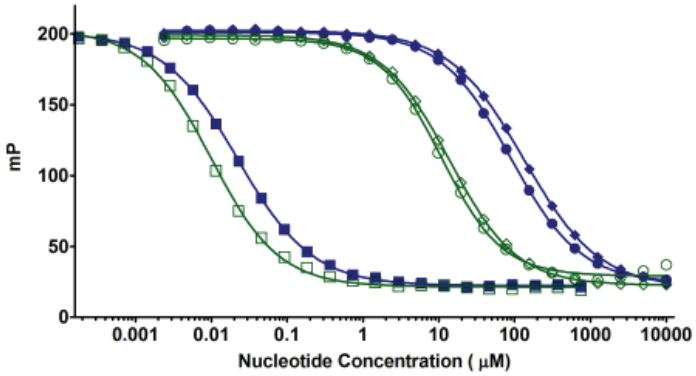
cAMP/AMP cGMP/GMP: Phosphodiesterases



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



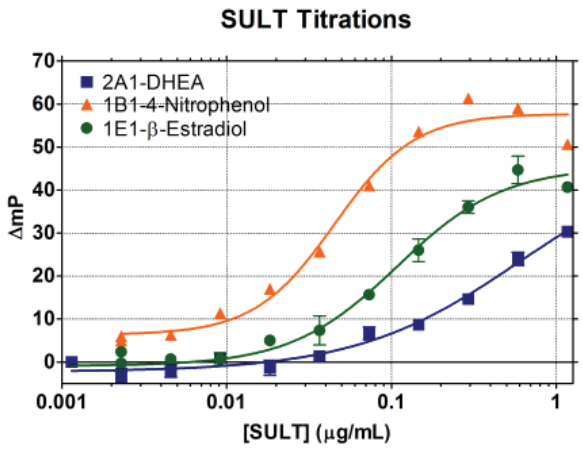




Transcreener reagents, antibodies and tracers, have been developed for detection of ADP, UDP, PAP, and AMP/GMP for screening respectively Kinases, Glycosyltransferases, Sulfotransferases and Phosphodiesterases. Note that there are applications outside these primary target families as well: e.g. ATPases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

Figure 3.

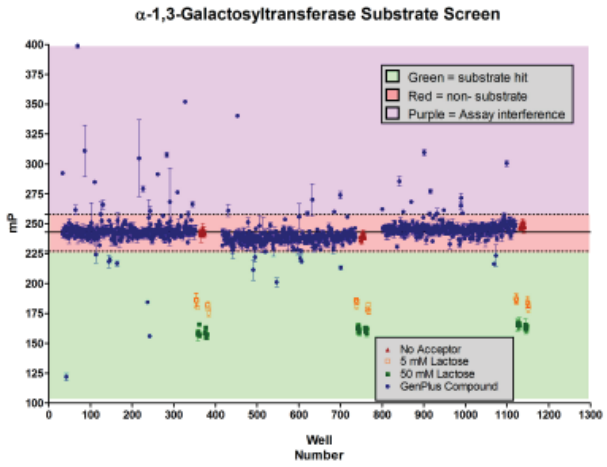
Transcreener™ Assay Platform Allows Detection of Purified Cytosolic SULT Isoforms

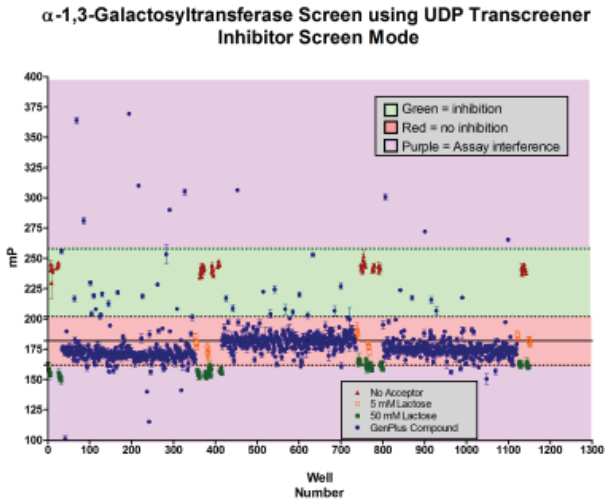


Homogenous immunodetection of phosphoadenosine phospate (PAP) enables detection of purified cytosolic SULT isoforms. Reactions contained 15 µM PAPS and the indicated acceptor substrates. Anti-PAP antibody and tracer were added at the start of the reaction and polarization values were read periodically to monitor product formation. The data shown is from the one hour timepoint.

Figure 4.

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



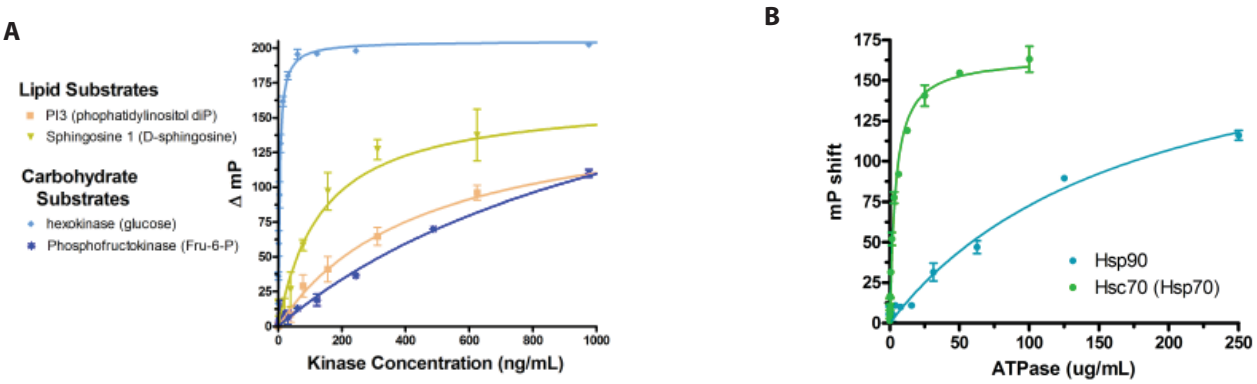


The Transcreener assay platform is unique in that it provides the means to screen for both substrates and inhibitors. GalT assays were performed in 30 µL volumes with 50 ng/mL alpha-1,3,-GalT, and 50 µM UDP–Gal. The enzyme used in this study was a His-tagged, recombinant alpha-1,3-GalT expressed in and purified from E. coli. The standard assay conditions were: 10 mM Tris-HCl pH 7.0, 10 mM $MnCl_2$, 1.25% v/v UDP Antibody, 2nM UTP-488 Alexa Fluor® tracer was synthesized in house. Reactions were incubated at 37°C for 1 hour followed by 0.5 hour incubation at room temperature. Polarization measurements were taken with a Tecan Ultra plate reader using an Ex_{485nm}/Em_{535nm} filter set at 30°C. 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.

An inhibitor screen of the same compound set used in the substrate screen in above. Compounds were tested for their ability to inhibit galactose conjugation of the acceptor substrate lactose. Product formation (UDP) results in a negative shift in the mP signal and inhibition results in a positive shift from the control wells containing 5 mM lactose, the substrate concentration at which 90% of the maximum assay signal is observed.

Figure 5.

Transcreener™ KINASE Assay Allows Screening of Lipid and Carbohydrate Kinases and ATPases



Detection of **A)** kinase activity with lipid and carbohydrate acceptors and **B)** ATPase activity (water serves as acceptor) 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 K_m 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.

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 SULT Assay enables detection of steroid and xenobiotic sulfation by cytosolic sulfotransferases.
- The Transcreener UGT Assay (UDP detection) allows screening for glycosyltransferase acceptors and inhibitors.
- The Transcreener Kinase Assay (ADP detection) can be used to detect any ATP-dependent phosphotransferase reaction, regardless of the acceptor substrate, including ATPases (H_20 acceptor).

Transcreener™ HTS Assay Platform is patent pending. Transcreenerr™ is a trademark of BellBrook Labs. Alexa Fluor® is a registered trademark of Molecular Probes, Inc (Invitrogen). This work was supported by NIH SBIR grant GM69258, GM59542 and CA110535

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