Development of a Homogenous Transcreener® UDP² TR-FRET Assay for High Throughput Screening of Glycosyltransferases

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Abstract

Glycosyltransferase enzymes participate in extremely diverse metabolic and regulatory roles by catalyzing the transfer of sugar molecules to protein, lipid and carbohydrate acceptors as well as endogenous and xenobiotic small molecules. Glycosyltransferases are increasingly of interest as targets for "substrate reduction therapy" in lysosomal storage diseases such as Gaucher and Fabry diseases, and as anti-microbial targets for disrupting bacterial cell wall biosynthesis. From an HTS assay perspective, they are a challenging target class because of the diversity of both donor and acceptor substrates. Most glycosyltransferase screening efforts have relied on either enzyme-coupled assay methods, which are prone to interference, or mass-spectrometry, which is not available to many labs. This poster describes the development, optimization and validation of the Transcreener UDP² TR-FRET assay. The assay produces a far-red, time-resolved Förster-resonance-energy-transfer (TR-FRET) signal that is less prone to interference from fluorescent compounds than other detection modes and can be used with any enzyme that produces UDP from a variety of sugar donors (UDP-Glucose, UDP-Galactose, etc.). The result is a simple, single step, homogenous assay amenable to HTS on any multimode plate reader. Signal stability, assay robustness and its universality will be demonstrated.

Transcreener UDP² TR-FRET Assay: Universal, Mix-and-Read Detection of UDP-Glycosyltransferases

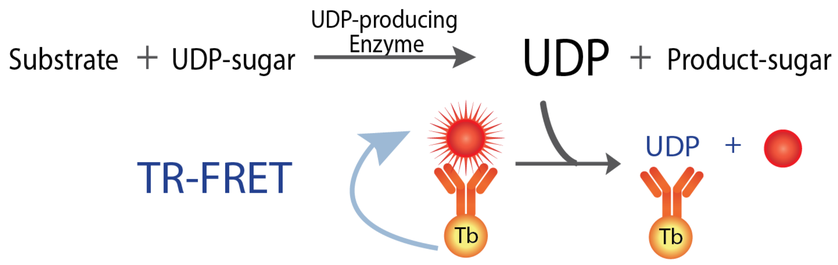
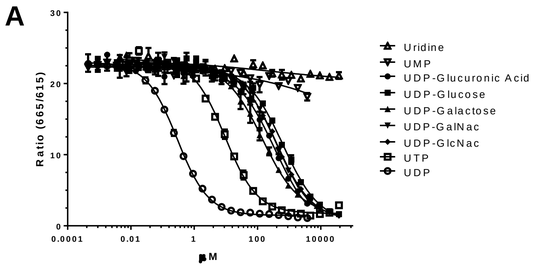


Figure 1. Transcreener UDP² TR-FRET Assay. The Detection Mixture comprises an UDP HiLyte 647 tracer bound to an UDP² antibody-Tb conjugate. Excitation of the terbium complex results in energy transfer to the tracer and emission at a higher wavelength after a time delay. UDP displaces the tracer, which causes a decrease in TR-FRET. The time gated nature of the detection method largely eliminates interference that can result from prompt fluorescence.

Antibody is Highly Selective for UDP vs. UDP-Sugars

A



B

	UDP	UTP	UDP-GA	UDP-Glu	UDP-Gal	UDP-GalNac	UDP-GlcNac	Uridine	UMP
IC_{50}, µM	0.3	9.3	345.8	638.7	143.1	401.6	402.1	nd	nd
Selectivity		32.3	1197	2210.8	495.3	1390.1	1391.8		

Figure 2. Competition Curves with Various UDP-Sugars. A. UDP-Sugars and uridine derivatives were titrated to demonstrate the selectivity of the UDP²-antibody-Tb for UDP. **B.** IC_{50} and selectivity values of the nucleotides demonstrate the ability of the antibody to either distinguish between UDP and other uridines or UDP-Sugars.

Overnight Reagent and Signal Stability

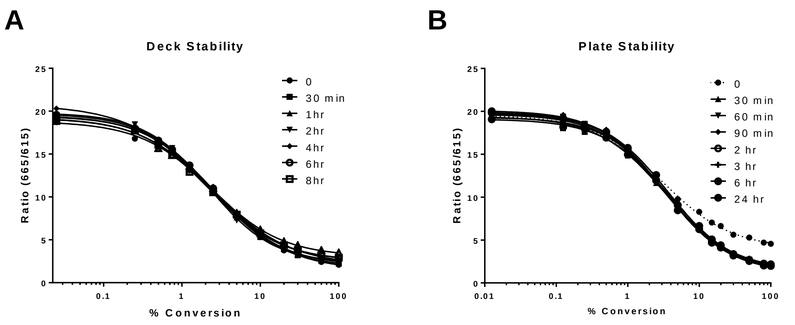
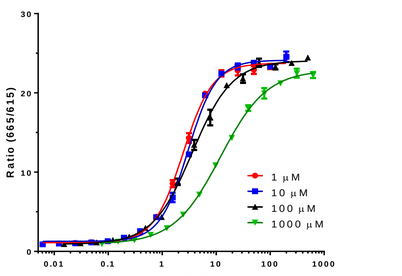


Figure 3. Reagent and Signal Stability of the UDP²- TR-FRET Reagents. A. Deck stability represents the ability of the reagents to remain functional when placed on the deck of an automated liquid dispenser. **B**. Plate stability demonstrates signal stability of reagents after being added to standard curve mixtures in an assay plate. Reagent and signal stability showed less than 10% change at 10% conversion up to 8 and 24 hours respectively with Z' values of 0.7.

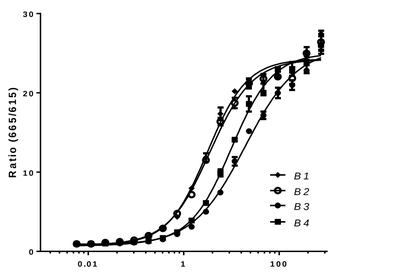
Tunable Assay Response and Dynamic Range

A



[UDP-Glucse], µM	1	10	100	1000
[UDP-HiLyte 647], nM	7.3	8.5	15.7	67

B



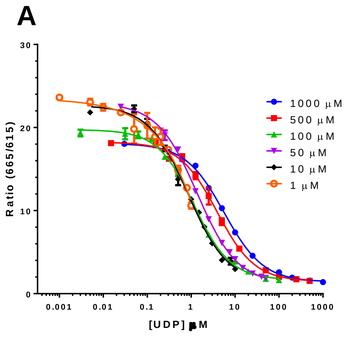
Enzyme Buffer	B1	B2	B3	B4
[UDP-HiLyte 647], nM	16.5	20.9	173.8	56.1

Figure 4. Optimizing UDP HiLyte647 Tracer concentration.. The UDP HiLyte647 Tracer was titrated with **A.** 1–1000 µM UDP-Glucose or **B.** in 4 diverse enzyme buffer systems. Typically an $EC_{60\text{-}85}$ will give a robust signal. Recommended [UDP HiLyte647 Tracer] shown below graphs.

B1) 100 µM UDP-GalNac, 10 µM EA2 peptide, 50 mM Tris, 2.5 mM $MnCl_2$
B2) 100 µM UDP-GalNac, 10 µM EA2 peptide, 25 mM Tris, 2.5 mM $MnCl_2$, 0.15 M NaCl, 2.5 mM $CaCl_2$.
B3) 400 µM UDP-Glucose, 25 mM Tris, 150 mM NaCl, 10 mM $MnCl_2$, 5 mM $CaCl_2$, 150 mM K_2SO_4
B4) 250 µM UDP-Galactose, 2.5 mM N-Acetyl-D-Glucosamine, 25 mM Tris, 150 mM NaCl, 10 mM $MgCl_2$, 10 mM $MnCl_2$.

Accommodates a Range of UDP-Sugar Concentrations

A



B

Z'	UDP-Glucose, µM			
% Conv	1	10	100	1000
0.05	---	---	---	0.2
0.5.	---	---	0.1	0.7
2.5	---	0.2	0.8	0.9
10	---	0.7	0.9	0.9
15	0.2	0.8	0.9	0.9
30	0.7	0.9	0.9	0.9
100	0.8	0.8	0.9	0.9
LLD (nM)	150	250	500	500

Figure 5. UDP-Glucose/UDP Standard Curves and Robustness. A. 1–1000 µM UDP-Glucose/UDP standard curves were performed. **B.** Sample Z' and Lower Limit of Detection(LLD) were calculated to demonstrate the robustness and sensitivity of the assay.

LLD is defined as the concentration of UDP the generates a Z' >0

$$Z' = 1 - \frac{[(3 \times 0\%\ \text{Conv. STDev}) + (3 \times x\%\ \text{Conv. STDev})]}{(\text{mean of 0\% Conv. Ratio}_{665/615} - \text{mean of x\% Conv. Ratio}_{665/615})}$$

Robust Initial velocity Detection with Diverse Glycosyltransferases, Substrates and Buffer Systems

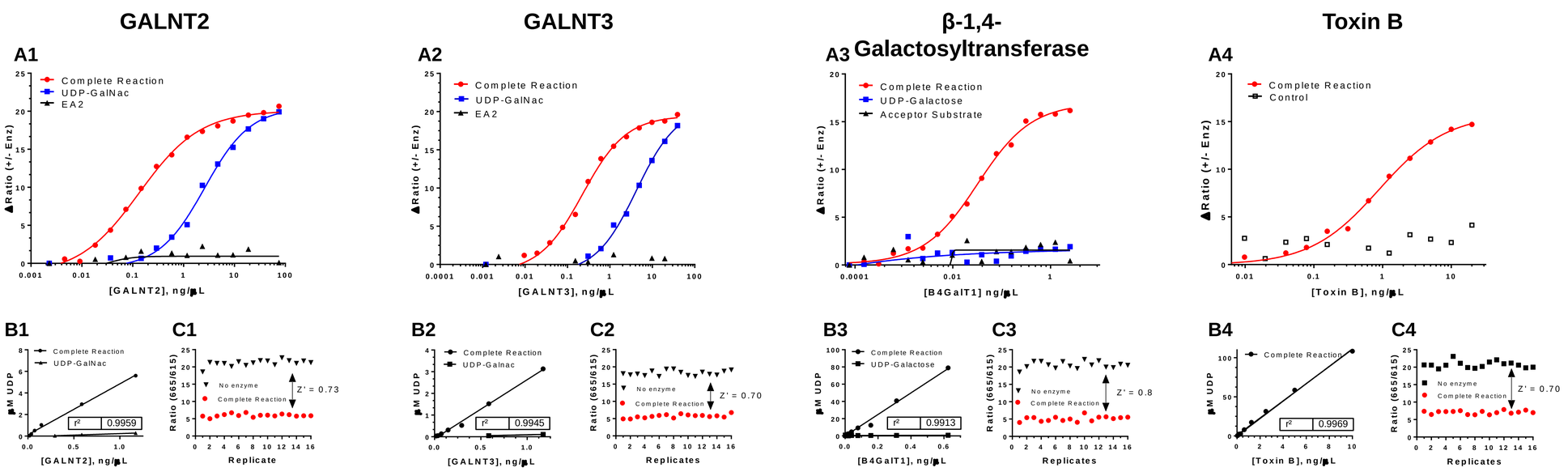


Figure 6. Enzyme reactions with a diverse set of donor and acceptor substrates. A(1-4). GALNT2, GALNT3, BGalT1 or Toxin B were titrated in buffers *B1, B2, B4, or B3* respectively *(*Fig 4). **B(1-4).** Raw data was converted to product formation to show a linear correlation of the enzyme with the product formed (UDP) confirming initial velocity conditions. **C(1-4).** Follow-up experiments (16 replicates) using the [EC_{80}] of the enzymes from **A(1-4)** were performed to determine Z'.

Conclusions

- ***UDP^2 TR-FRET Assay is a homogenous, direct, single addition mix and read assay.***
- ***The assay is extremely sensitive and robust***
- ***The assay can be used for screening and profiling glycosyltransferases with different UDP-Sugars and substrates***
- ***The Transcreener® HTS assay technology has been validated in over 50 million wells of screening.***

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