Homogeneous Detection of Glycosyltransferase Activities with Universal Bioluminescent Assays

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1. Introduction

Traditional assays for Glycosyltransferase (GT) activity are not easily configured for rapid GT activity detection nor for high throughput screening; they rely on a) cumbersome detection methods such as detection of radiolabeled substrate requiring product isolation, b) non-homogeneous antibody-based assays or c) non-plate-based format (i.e., HPLC or mass spectrometry).

Glycosylating enzymes use nucleotide-sugars as substrates (e.g., UDP-Gal, GDP-Fuc and CMP-Sialic acid), and in a glycosyltransferase reaction, the nucleotide moiety is released as a product after sugar transfer. Therefore, an assay that detects the nucleotide molecule could be generically used to assess all glycosyltransferases activity in vitro.

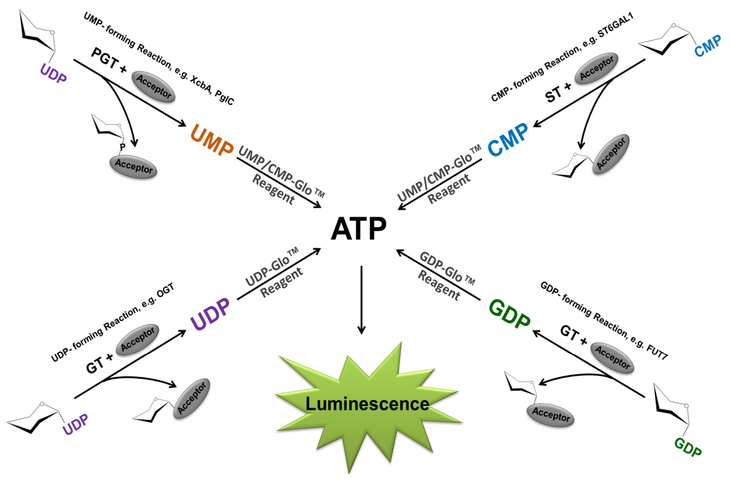
We developed four bioluminescent assays for measuring GT activities based on UDP, GDP, UMP and CMP quantification. These assays have the following features:

- One-step detection: After GT reaction, the nucleotide product simultaneously converted to ATP, then to light in a robust luciferase reaction.
- The light output is proportional to the nucleotide concentration ranging from low nM to 25-50µM.
- Very sensitive and robust assays, two highly desirable and essential features required for measuring the activity of the majority of GT classes.
- Simple assays, do not require antibodies, nor modified substrates.

The development of these nucleotide detection assays (UDP-Glo, GDP-Glo and UMP/CMP-Glo) will enable the investigation of a large number of GTs and may have significant impact on diverse areas of glycobiology research.

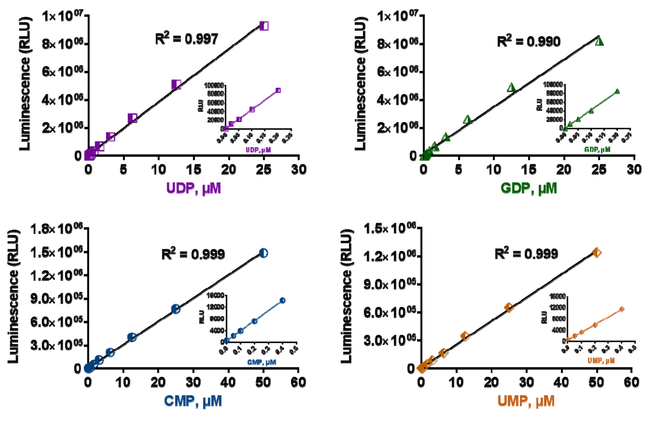
2. Principle and Format of Glo Assays for UDP, GDP, UMP and CMP

Simple "Add and Read": No radioisotopes. No product separation. No HPLC



- One Step Detection: After the GT reaction, the detection reagent is added in 1:1 ratio.
- Luminescence signal is recorded after 60min incubation.
- Luminescence is proportional to the nucleotide produced and to the GT activity.

3. Assay is Linear and Sensitive; Signal is Stable

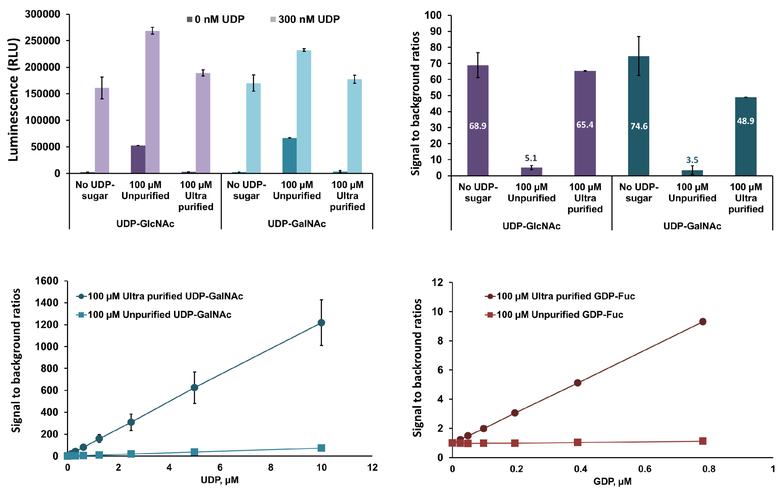


Nucleotide	Assay	Signal to Background ratios (fold) at each nucleotide concentration (µM)											
		25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0.02	0
UDP	UDP-Glo	12368	6803	3588	1828	917	459	227	119	60	30	16	1
GDP	GDP-Glo	41700	24917	13317	7028	3533	1788	898	436	208	110	54	1
		50	25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0
UMP	UMP/CMP-Glo	1922	1009	535	259	139	68	34	18	9	5	3	1
CMP		2186	1128	595	308	166	83	40	21	11	6	3	1

- Bioluminescent nucleotide assays are linear up to 25-50µM with high dynamic range.
- The assays can detect nucleotide concentration as low as 10nM with > 2-fold S:B.
- The bioluminescent signal generated from the assays is stable over time allowing batch processing in HTS.

4. Effect of Nucleotide-Sugar Quality on Detection and Assay Sensitivity

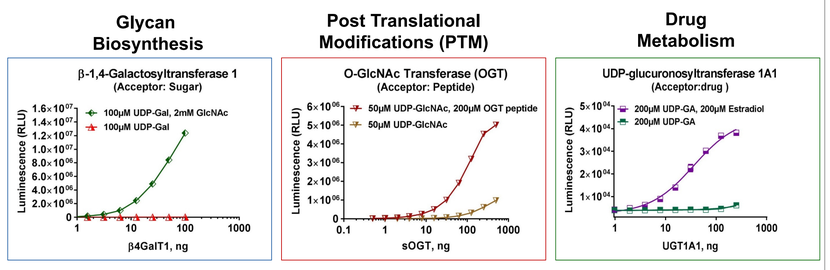
Comparing commercial and purified nucleotide-sugars in UDP or GDP detection assay



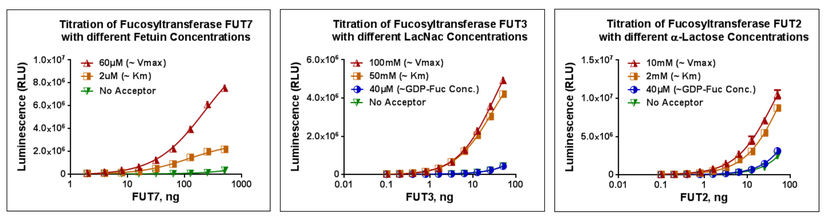
- Commercial Sugar donors contain free nucleotides thus decreasing UDP, GDP, or CMP detection sensitivity.
- Assays sensitivity is recovered using Ultra-Pure sugar donors.

5. Glo Assays for UDP, GDP and UMP/CMP are Universal for Most Glycosyltransferases

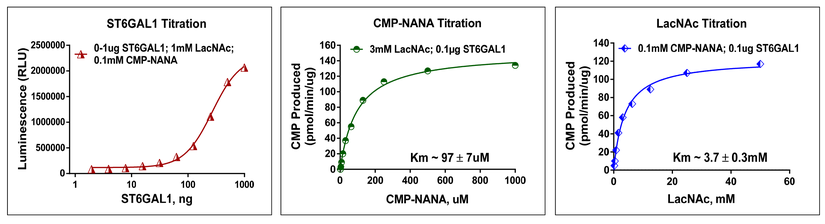
UDP-Glo is one assay for diverse glycosyltransferase-substrate combinations



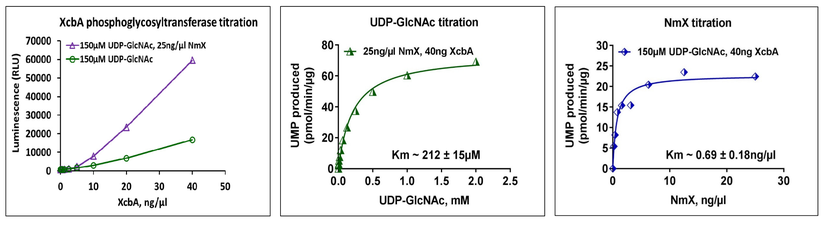
Optimization of Fucosyltransferase acceptor substrate concentrations using bioluminescent GDP-Glo assay



Biochemical characterization of ST6GALT1 using bioluminescent UMP/CMP-Glo assay

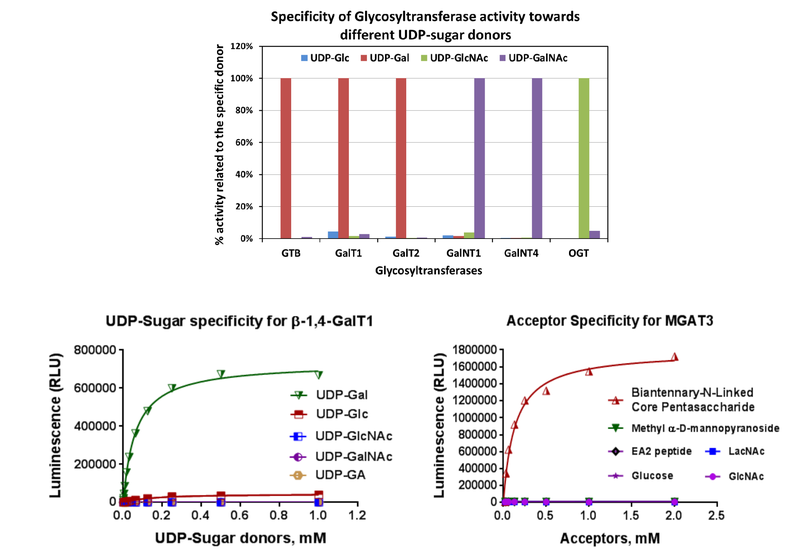


Biochemical characterization of N-acetylglucosamine-1-phosphotransferase (XcbA) using bioluminescent UMP/CMP-Glo assay



- The bioluminescent platform detects the activity of any nucleotide-sugar using GT regardless of substrate chemical structure.
- Bioluminescent GT assays are used to determine biochemical values for different sugars by diverse GTs.
- Bioluminescent GT assays are very sensitive and allow detection of acceptor-dependent and -independent nucleotide-sugar hydrolysis.

6. Profiling GT Substrate Specificity Using Bioluminescent Detection

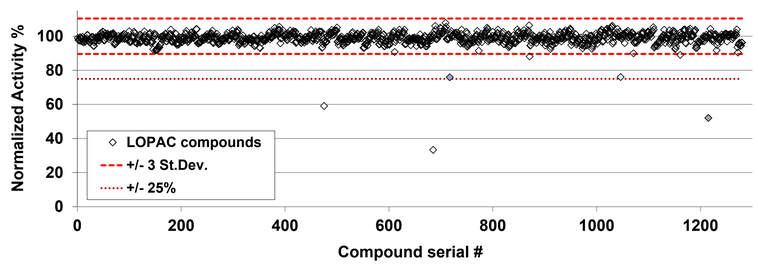


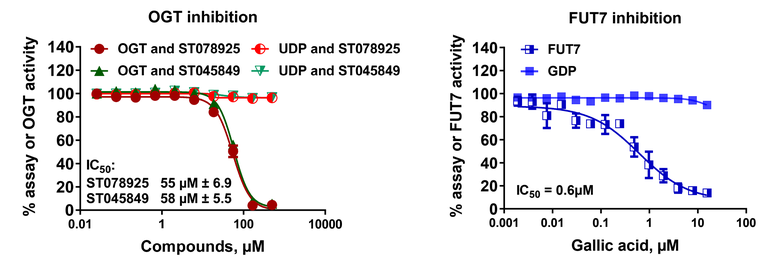
Bioluminescent nucleotide assays can be used to:

- Study specificity of transfer of different sugars by diverse GTs.
- Find specific sugar acceptor substrates for GTs.

7. Glycosyltransferase Inhibitor Studies Using Bioluminescent Detection Assays

Screening UDP-Glo Reagents for compound interference with LOPAC library
(5µM UDP in 5µl reaction)





- Bioluminescent GT assays are robust and resistant to chemical compound interference.
- Bioluminescent GT assays can detect accurately inhibition of GTs by known selective compounds.

8. Conclusions

Various applications of the UDP-Glo, GDP-Glo and UMP/CMP-Glo nucleotide detection assays were presented here, including studies on specificity of transfer of different sugars to different acceptors by diverse GTs, and screening for specific GT inhibitors along with the study of their mode of action.

Bioluminescent Nucleotide Detection Assays have the following advantages:

- Are universal. The assays can be used for all glycosyltransferases that have a nucleotide as a substrate.
- Highly sensitive assays that allow the detection of low activity GTs or use of low amounts of purified GTs.
- Easy to use assays. One step addition and read.
- Detection reagents are resistant to chemical interference, making the assays Ideal for GT inhibitor screening.
- HTS friendly: sensitive in low volume format and stable signal in batch processing.
- Bioluminescent UDP, GDP and UMP/CMP detection is adequate for studying acceptor and donor substrates for any GT.

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