Screening for Glycosyltransferase Activity Inhibitors With Homogeneous Bioluminescent Nucleotide Detection Assays

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1. Introduction

Glycosyltransferases (GTs) play a pivotal role in a multitude of biological processes such as cell-cell communication, immune responses, cell signaling and epigenetic regulation of gene expression. Scientists studying diabetes, inflammation and infectious diseases, for example, find GTs to be attractive drug targets. As this class of enzymes is important, there is strong need for biochemical assays to monitor their activities and modes of regulation, and to search for selective and potent inhibitors. Traditional assays for GT activity analysis are not easily configured for rapid or high throughput detection because they rely on cumbersome technologies such as radiometry.

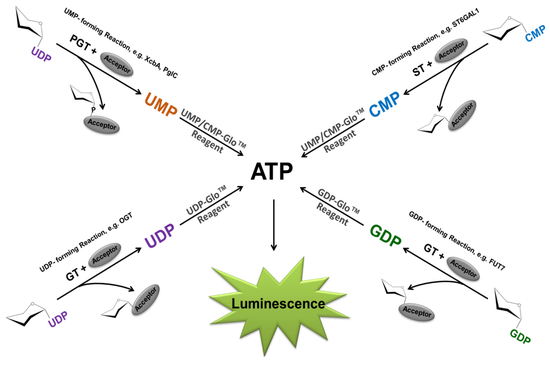
In a typical glycosyltransferase reaction, after sugar transfer from the donor nucleotide-sugar substrate, the nucleotide moiety is released as the reaction product. Therefore, an assay that detects the nucleotide molecule could be used to assess all glycosyltransferases activity in vitro.

We developed four bioluminescent assays for measuring GT activities based on nucleotide quantification. These assays have the following features: a) One-step detection. b) The light output is proportional to the nucleotide concentration ranging from low nM to 25-50µM. c) Very sensitive and robust assays. d) Simple assays, do not require antibodies, nor modified substrates.

The development of these nucleotide detection assays will enable the investigation of a large number of GTs and may have significant impact on diverse areas of glycobiology research.

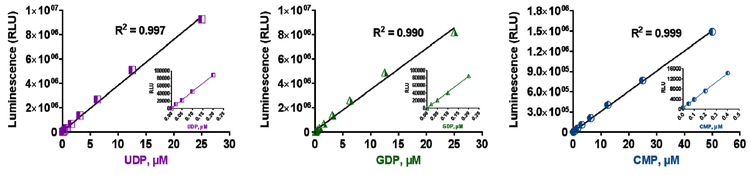
2. Principle and Format of UDP, GDP, UMP and CMP Glo Assays

Principle: Nucleotides are Converted into ATP that is Detected via Bioluminescence



- 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.
- No radioisotopes. No product separation. No HPLC

3. Assays are Linear and Sensitive; Signal is Stable



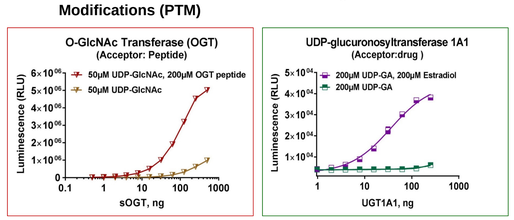
Nucleotide	Assay	Signal to Background ratios (fold) at each nucleotide concentration (µM)											
		25	12.5	6.25	3.13	1.56	0.78	0.39	0.20	0.10	0.05	0.02	0
UDP	UDP-Glo	12368	6803	3588	1828	917	459	227	119	60	30	16	1
GDP	GDP-Glo	41700	24917	13317	7028	3533	1788	898	436	208	110	54	1
		50	25	12.5	6.25	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.

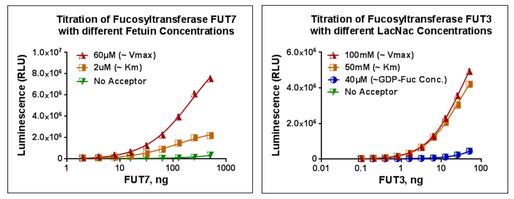
4. Glo Assays for UDP, GDP and UMP/CMP are Universal for Most Glycosyltransferases

UDP-Glo is One Assay for Diverse Glycosyltransferase-Substrate Combinations

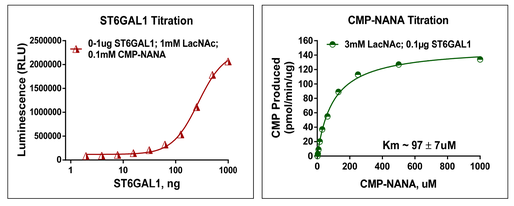
Post Translational Modifications (PTM) | **Drug Metabolism**



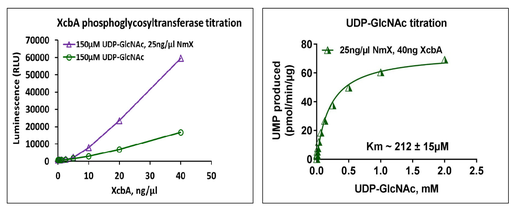
Optimization of Fucosyltransferase Acceptor Substrate Concentrations Using Bioluminescent GDP-Glo Assay



Biochemical Characterization of ST6GAL1 Using Bioluminescent UMP/CMP-Glo assay

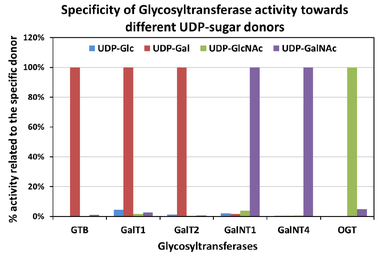


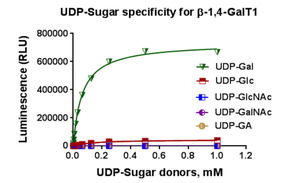
Biochemical Characterization of N-Acetylglucosamine-1-Phosphotransferase (XcbA) Using Bioluminescent UMP/CMP-Glo Assay

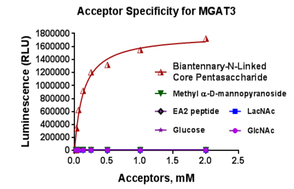


- The bioluminescent platform detects the activity of any nucleotide-sugar using GT regardless of substrate chemical structure.
- Bioluminescent GT assays are used to determine biochemical values for different sugars by diverse GTs.
- Bioluminescent GT assays are very sensitive and allow detection of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

5. Profiling GT Substrate Specificity Using Bioluminescent Detection





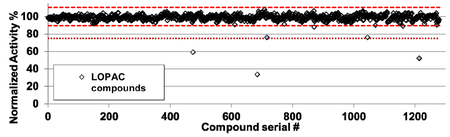


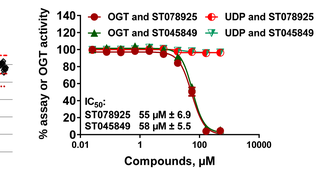
Bioluminescent nucleotide assays can be used to:

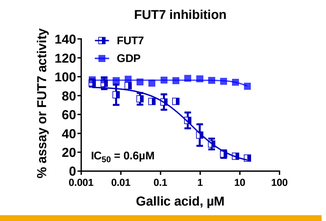
- Study specificity of transfer of different sugars by diverse GTs.
- Find specific sugar acceptor substrates for GTs.

6. 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.

7. 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 were used with diverse Glycosyltransferase families regardless of their substrate chemical structures
- Highly sensitive assays that allowed the detection of low activity GTs or the 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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