Identification of Potential Small-Molecule activators of Glucokinase (GCK)

Flor De La Cruz,
Mentor: Amedeo Veter
PI: Bridget Wagner
Broad Institute
Chemical Biology
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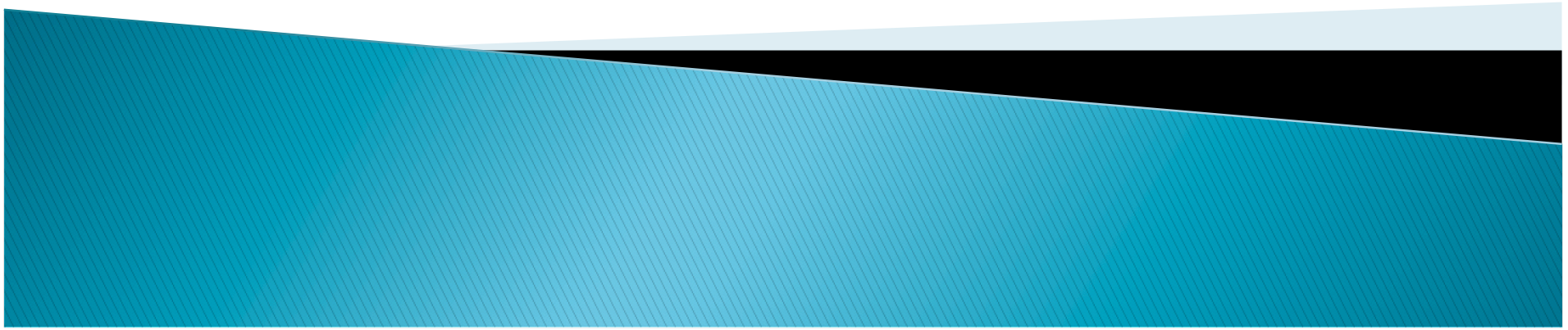
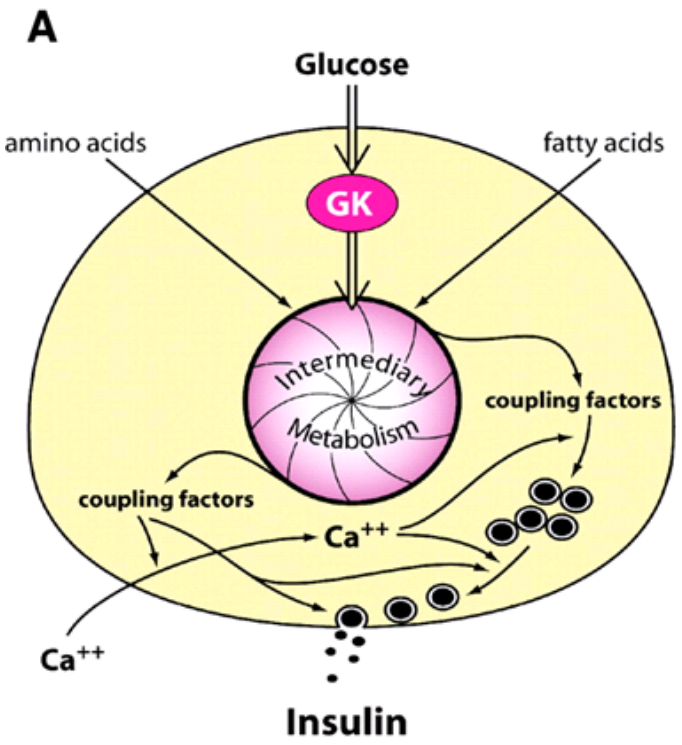


Glucokinase

- Glucokinase is a member of the hexokinase enzyme family and is found in the pancreas
- It acts as a glucose sensor in the glycolysis pathway;
- It is speculated that it might represent a `diabetes gene';
- More recent findings show that it acts:
  - sensor in the secretion of insulin by beta cells
  - can induce beta-cell proliferation



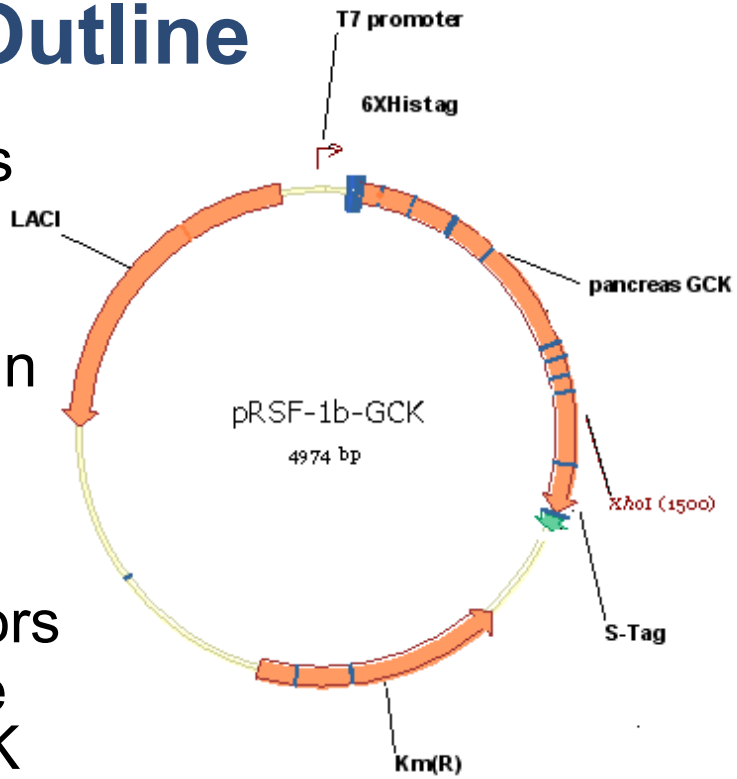
From: Matschinsky, F. et al
Diabetes 2006 55:1-12

AIM

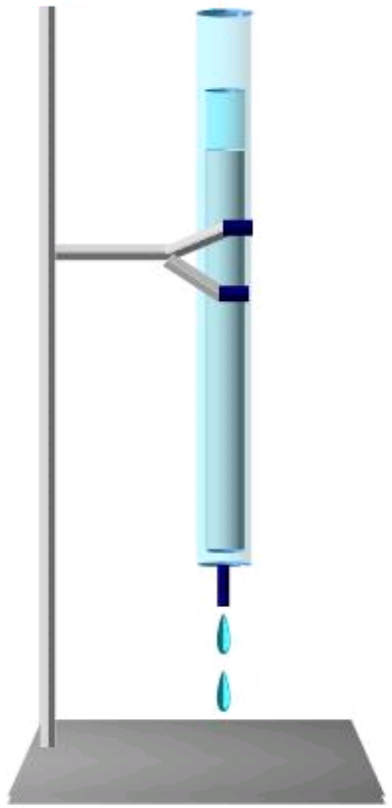
Find new small molecule activators of GCK

Experimental Outline

- RNA extraction from mouse islets
- Reverse Transcription and PCR
- Clone GCK cDNA into pRSF-1b
- Express recombinant GCK protein
- Develop an *in vitro* kinase-glo luminescent assay system
- Assay enzyme activity with and without commercial GCK activators
- Screen the Broad small molecule chemical library for potential GCK activators



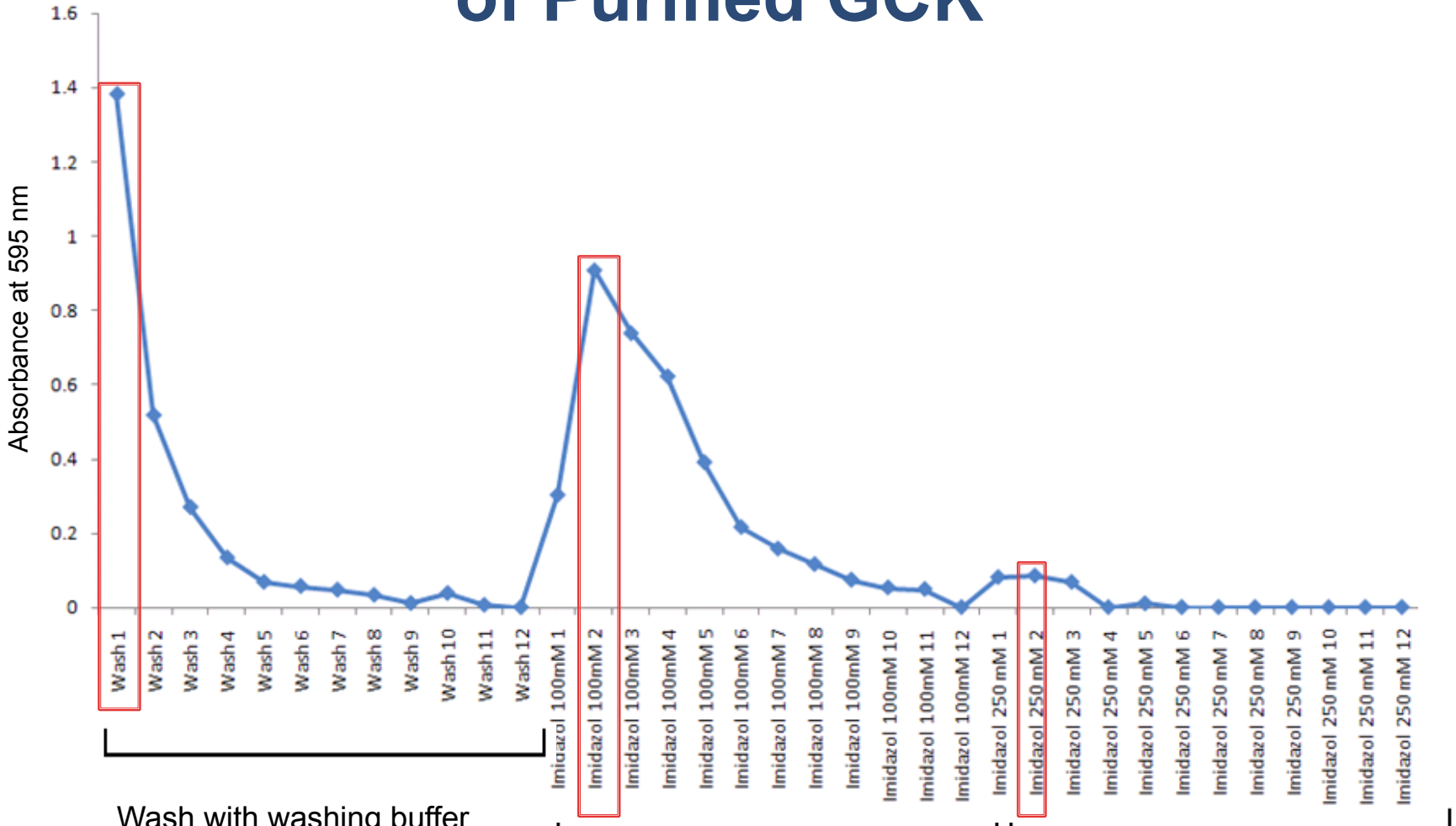
Steps for Purification of GCK



1. 1 ml of Ni-NTA equilibrated

2. Flow through washed with:
   - -- 1mL of washing buffer 12 times
   - -- 1mL elution buffer containing 100mM Imidazol, 12 times
   - -- 1mL elution buffer containing 250mM Imidazol,12 times

Protein Assay with Samples of Purified GCK



Wash with washing buffer
Samples1-12

Elution with 100mM Imidazol
Samples 1-12

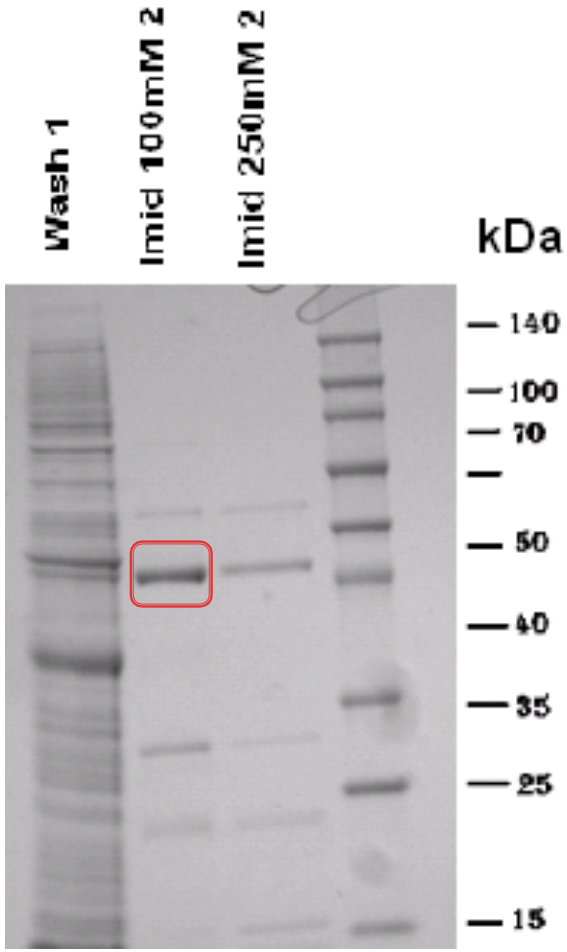
Elution with 250mM Imidazol
Samples 1-12

Gel showed a clean protein

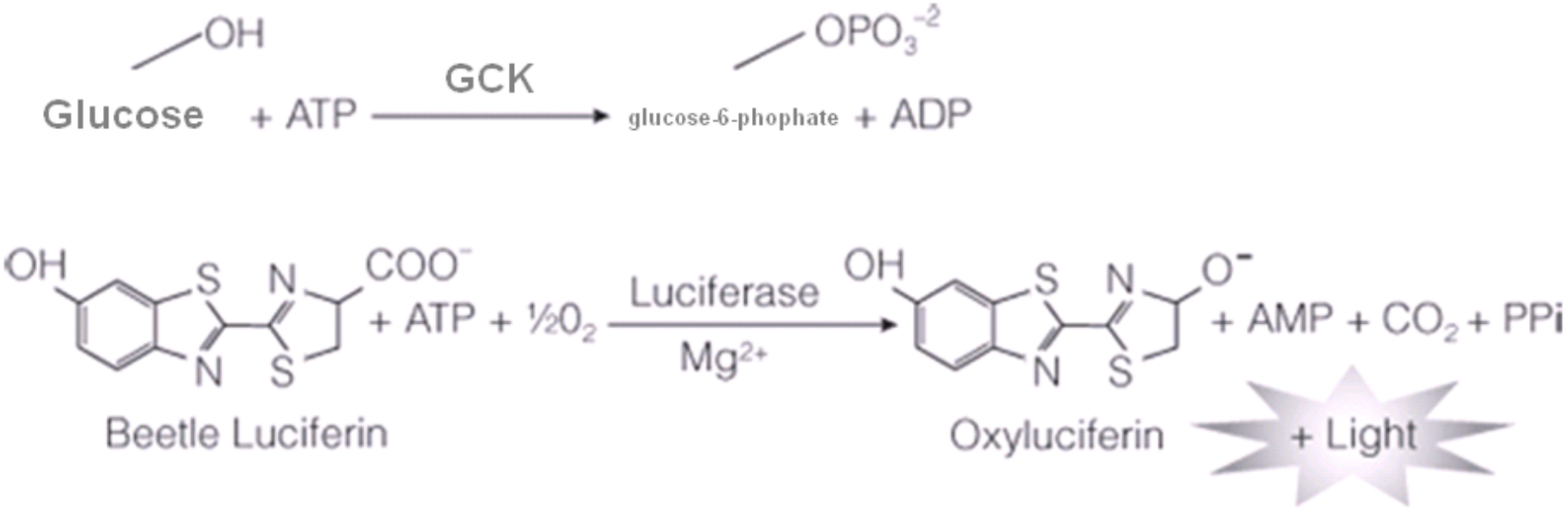
Gel-3 Samples

Sample 1: Wash 1
Sample 2: Imidazol 100mM 2
Sample 3: Imidazol 250mM 2

- Further purification was not needed



Schematic Representation of Kinase-Glo Luminescent Assay

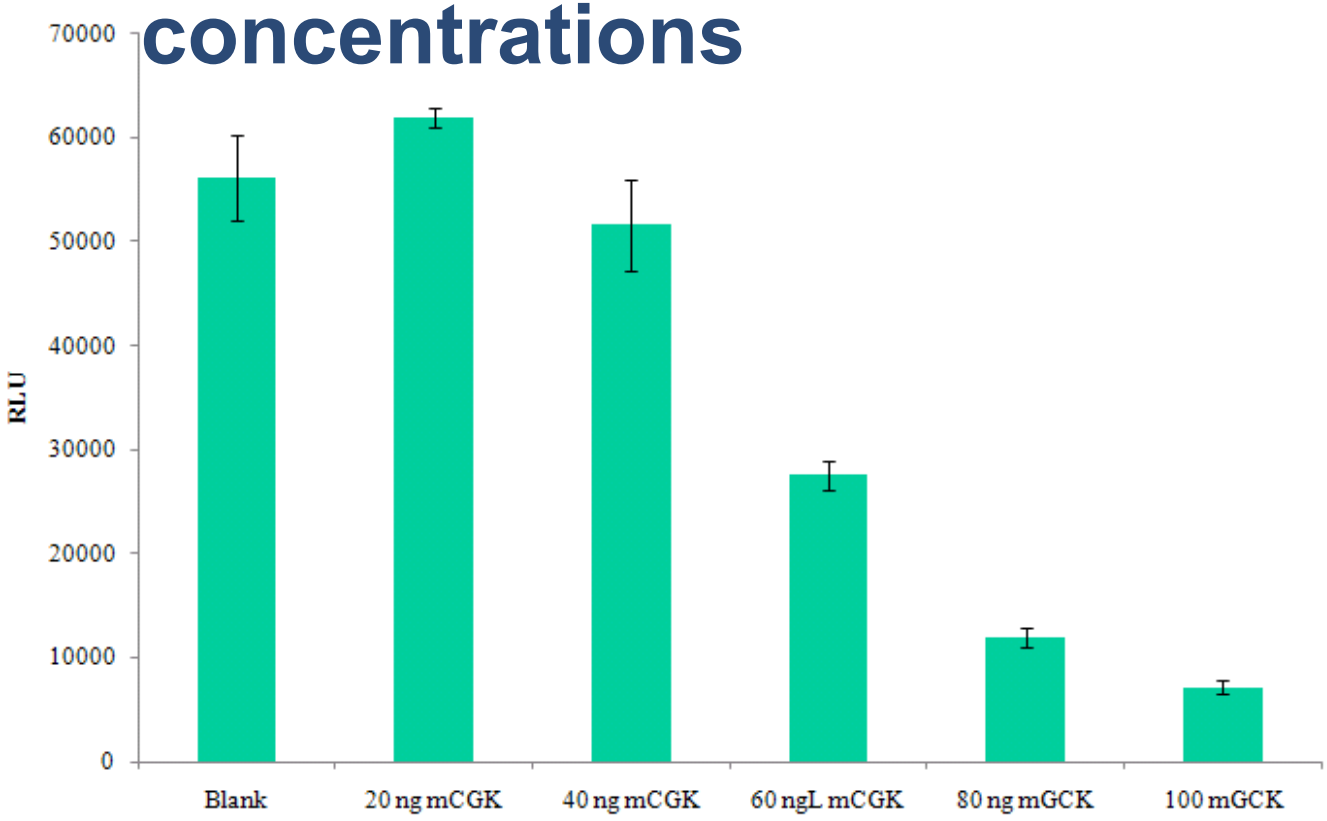


From: Promega Technical Bulletin; Kinase-Glo Luminescent Assay Platform TB372

Luminescent Assay with varying GCK concentrations

Assay

Enzymatic Assay meausuring ATP consumption by GCK at concentrations ranging from 20 ng to 100 ng per well

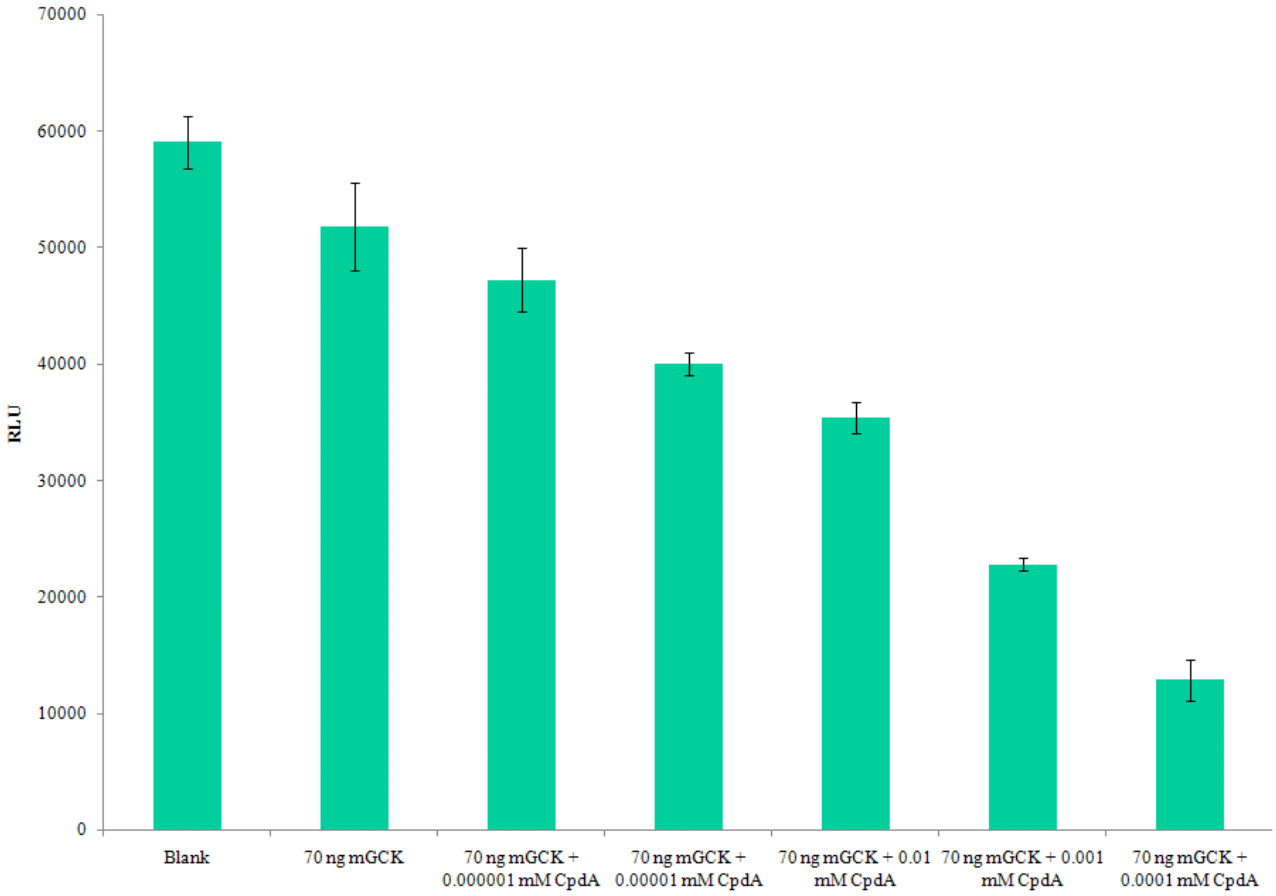


Luminescent Assay with GCK Activator CpdA

ASSAY

- Optimized Enzymatic Assay with GCK Activator CpdA at differnet concentrations ranging from 1 nM to 10 uM

- Z' factor of 0.3 using 70 ng of GCK per well in absence or in presence of 1 mM CpdA.



Conclusion

- In conclusion we developed an assay that appears suitable for use in high-throughput screening of novel GCK activators

- Now we can proceed with high-throughput screens of the Broads chemical library

Acknowledgements

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