Monitoring of insulin granule packaging in live cells using homoFRET-FP detection

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- Within the tightly packed confines of insulin granules homoFRET can occur and changes in polarization signal can be detected
- FP measurements in live cells using the PHERAstar® microplate reader

Introduction

Diabetes mellitus is characterized by disruption of normal metabolism that stems from resistance to insulin or poor insulin secretion1. In 2014 it was reported that nearly 10% of the U.S. population suffers from diabetes and that 90% of diabetes cases are type-2 diabetes2,3. Because of the high prevalence of this disease and the equally high financial burden associated with treatment it remains a focus to find new therapeutics.

Secretion of physiologically active insulin is regulated at multiple steps as outlined in Figure 1. Each of these steps represents a chance for therapeutic intervention4.

Assay Principle

This application note describes a high-throughput screening compatible cell-based assay that uses a preproinsulin-mCherry (PPI-mCherry) system. The application exploits the fact that when a fluorophore is at a high local concentration FRET can occur between the same type of fluorophores. This phenomenon is called homoFRET (HF). Furthermore if polarized light is applied as the excitation light it will become randomized as the HF occurs between adjacent fluorophores. It was reasoned that a HF-FP approach would be suitable to monitor the extent of packing of mature insulin into dense core granules (Figure 2).

Materials & Methods

- 384-well black/clear bottom plates (NUNC #152029)
- Rat insulinoma (INS-1) cells were donated by Christopher Newgard at Duke University
- Preproinsulin (PPI) mCherry Reporter Construct was made in Dr. Brenman’s lab at UNC
- 1,280 molecule FDA-approved drug set (Prestwick Chemical Library)
- 502 purified natural products (Enzo Life Sciences)
- PHERAstar microplate reader from BMG LABTECH

INS-1 cells transfected with PPI-mCherry were grown for 48 hours and then exposed to the indicated concentrations of agonists/antagonists for 4 hours.

PHERAstar instrument settings

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Results & Discussion

To validate the homoFRET-FP approach cells were treated with Bafilomycin, a vacuolar-type H+ ATPase (V-ATPase) inhibitor known to block vacuole maturation and thus block insulin granule formation (Figure 3). The results show that increasing concentrations of Bafilo-
mycin result in an increase in mP value correlating with decreased granule formation.

Figure 5 shows the representative confirmation of the three hits from the pilot screen. Overall the screen exhibited a hit rate of 1.4 % and a confirmation rate of 36.4 %.

For subsequent experiments 83 nM Bafilomycin served as a positive control compared to DMSO negative control. Pilot screening was performed using 2 different compound libraries. The results from these experiments showed a Z’-factor that indicates the assay is indeed suitable for HTS. Furthermore, 26 compounds were shown to be active [Figure 4].

Conclusion

These results establish a novel cell-based FP biosensor to identify compounds that modify insulin granule packaging. This technology may serve as a new method for assessing protein-protein interactions in live cell systems.

References