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

N.Y. Yi[1], Q. He[1], T.B. Caligan[1], G.R. Smith[1], L.J. Forsberg[2], J.E. Brenman[2] and J. Z. Sexton[1]
[1]North Carolina Central University, Durham, NC [2]UNC Chapel Hill School of Medicine, Chapel Hill, NC

- **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 secretion[1]. 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 diabetes[2,3]. 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 intervention[4].

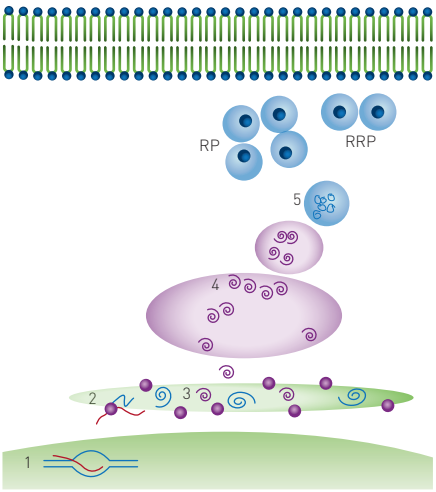

Fig. 1: Insulin production and storage. Key biological steps in insulin production: 1) Transcription, 2) translation and translocation to the endoplasmic reticulum, 3) folding and signal peptide cleavage 4) Golgi transport and packaging into secretory vesicles 5) cleavage to produce mature insulin. Mature insulin is stored in dense-core granules in two populations: RRP = ready releasable pool and RP = reserve pool.

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).

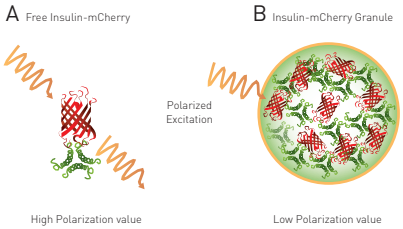

Fig. 2: homoFRET-FP to detect packaging of insulin in dense core granules in live cells. A) Free insulin-mCherry with polarized excitation will exhibit conserved polarization and relatively high MP signal. B) Within dense core granule polarized light will exhibit homoFRET, randomized polarization and a decrease in MP signal[4].

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

Measurement type:	Fluorescence Polarization
Measurement mode:	End point
Optic module:	FP(590-50/675-50/675-50)
Gain:	adjusted prior to test run
Target mP value:	400
Focal height:	7.2
Flashes / well:	200

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 Bafilomycin result in an increase in mP value correlating with decreased granule formation.

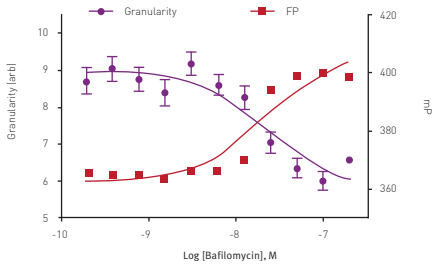

Fig. 3: Dose response to Bafilomycin in Insulin Granule Packing Assay. Analysis show an anti-correlation between homoFRET-FP signal and mCherry granularity.

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).

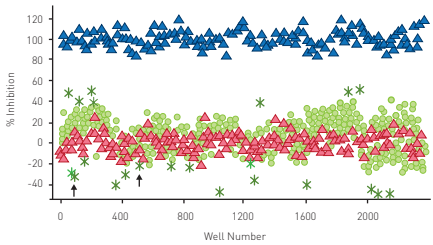

Fig. 4: FP data from pilot screen. Scatter plot of 1,782 test compounds (●) [hits (✱)], negative control (DMSO ▲) and positive control (Bafilomycin ▲). Arrows indicate Antimycin A1 is selected from both libraries.

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 %.

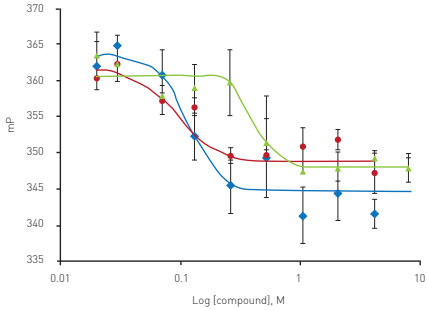

Fig. 5: Dose-response confirmation of active compounds. Oligomycin A (◆); EC_{50} = 0.114 µM, Antimycin A1 (●); EC_{50} = 0.089 µM, Rotenone (▲); EC_{50} = 0.37 µM. Adapted from Yi et al.[4]

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

1. Rorsman P *et al.* (2000) The Cell Physiology of Biphasic Insulin Secretion. *News Physiol. Sci.* **15**:72-77.
2. Centers for Disease Control and Prevention (CDC) (2014) National Diabetes Statistics Report.
3. Huang CJ *et al.* (2007) High expression rates of human islet amyloid polypeptide induce endoplasmic reticulum stress mediated beta-cell apoptosis, a characteristic of humans with type 2 but not type 1 diabetes. *Diabetes* **56**(8): 2016-2027.
4. Yi NY *et al.* (2015) Development of a Cell-Based Fluorescence Polarization Biosensor Using Preproinsulin to Identify Compounds That Alter Insulin Granule Dynamics. *Assay Drug. Dev. Technol.* **13(9)**: 558-569.

PHERAstar® *FSX*
PHERAstar® *FS*