Biomultisence Resonance Energy Transfer for High-Content Imaging

Angelika Foitzik1, Regis Grailhe2, Frauke Haenel3, Stefan Letzsch1, Joe Trask3, Alexander Schreiner1
1PerkinElmer, Hamburg, Germany; 2Institut Pasteur Korea, Korea; 3PerkinElmer, Hopkinton, MA, USA

Abstract

Fundamental processes in living cells are controlled by proteins, often acting through protein-protein interactions (PPIs), with other protein partners. Alterations in PPIs are linked to many diseases and are of increasing interest as potential drug targets. However, analyzing PPIs is challenging as it is drug screening for small molecules inhibiting PPIs. One method that allows PPIs to be quantified is Biomultisence Resonance Energy Transfer (BRET) which, in contrast to Förster Resonance Energy Transfer (FRET), does not require donor photo-excitation, circumventing autofluorescence and phototoxicity issues. A drawback of luciferases currently used for BRET experiments is their relatively low biomultisence-intensity, making image-based approaches challenging. Here, we present a high-content imaging application of BRET experiments using the Operetta CLS™ high-content analysis system and Harmony high-content imaging and analysis software. Nanolucerase (NLuc) was used as donor in combination with YFP as acceptor.

Serum Concentration Affects Luciferase Signal

To test the effect of serum concentration on luciferase signal, HeLa cells were reverse transfected with NLuc in CellCarrier® 384 Ultra Microplates (PerkinElmer) using jetPEI® (Polyplus-transfection) according to the manufacturers protocol. 24h post transfection, cells were imaged on an Operetta CLS high-content analysis system (PerkinElmer) using a 20x water immersion objective. Images from one field per well were acquired every 4min over a total time of 1h using a 430-500nm filter and LED power set to 0%. Exposure time was 10s and the final serum concentrations in growth medium were 1%, 2%, 5% and 10%. NLuc substrate concentration was 1x (NanobRET™ Nano-Glo® Detection System, Promega). Images were segmented and analyzed using Harmony software.

Intramolecular BRET

The data presented in figure 1 shows that NLuc can easily be imaged on an Operetta CLS system over a time course of 1h. To test whether the system could be used for BRET imaging, HeLa cells were transfected with either NLuc or a YFP-NLuc fusion protein as an example of intramolecular BRET. The plasmids used here are the same as in the study published by Jiho Kim and Regis Grailhe. Traditionally, BRET assays are analyzed using plate readers. Thus, we also measured BRET ratios on the 1x-YFP-NLuc system on the PerkinElmer VICTOR Nivo™ multimode microplate reader. Transfected cells were incubated with NLuc substrate at a final concentration of 1x and luminescence and BRET signals were collected in bottom read mode every 2min with an integration time of 1s over a total time course of 1h.

Figure 1: Higher serum concentrations negatively affect the NLuc signal intensity. HeLa cells were reverse transfected with NLuc and serial serum concentrations of 1%, 2%, 5% and 10% were tested. (A) Representative images of the time series. “0” indicates the images acquired directly after substrate addition while “60” indicates the images acquired after 60 min. The two black images before “0” were acquired before substrate addition. (B) Analysis of NLuc intensity shows that at 5% and 10% the NLuc intensity starts at a much lower value and decays rapidly. Based on this data 1% serum was used for all following experiments. Error bars represent one standard deviation, n=3.

Figure 2: BRET ratios calculated from the VICTOR Nivo multimode reader confirm the results obtained on the Operetta CLS system. The NLuc intensity decays curves (left panel) are very similar to those obtained on the Operetta CLS system (Figure 3 and 4). Additionally, the calculated BRET ratios (right panel) are constant over the time course. Untransfected HeLa cells (grey dots), NLuc only (blue dots) and YFP-NLuc (green dots). Each dot represents results from a single well and time point.

BRET assays analyzed on plate readers are based on the luminescence/fluorescence intensity values of whole wells. On high-content screening systems, individual cells, i.e. cells expressing the transfected plasmids, can be segmented. Hence, it is possible to determine local background intensities from image regions that do not contain expressing cells. From these areas, background signals can be determined and then be used to correct the BRET and luminescence signals to obtain background corrected BRET ratios.

Figure 3: High-content imaging and analysis enables single cell segmentation allowing for background correction of the BRET and NLuc signals. HeLa cells expressing NLuc-YFP were segmented (left image) and this region can be used as a restrictive mask to define foreground and background regions (right image).

Reference

Nanolucerase Signal Brightness Using Furimazine Substrates Opens Biomultisence Resonance Energy Transfer to Widefield Microscopy
Jiho Kim and Regis Grailhe

In this study, we found that serum-reduced medium improved the NLuc signal and the time series studies revealed that, despite the decay of biomultisence signal over time, the BRET ratio remained stable especially with background correction. This was observed for both intra- and intermolecular BRET experiments. The acquisition of a whole 96-well plate would take between 20-30 min depending on exposure time, therefore within the tested time frame a whole 96-well plate could easily be imaged. It should even be possible to extend the imaging time with the use of a 488nm excitation wavelength which is not supported by the BRET system but could improve the sensitivity of high-content imaging systems such as the Operetta CLS equipped with automated water immersion high NA objectives in combination with sensitive low noise cameras enable live cell BRET experiments in a 96-well plate format and robust PI quantification. These results show that BRET imaging assays may be used in future for secondary drug screening assays.

5 Summary

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