

Homogeneous Bioluminescence Resonant Energy Transfer for High Density Array on Imaging Chip

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ABSTRACT

A new method of bioluminescence resonance energy transfer (BRET) has been investigated using a CCD camera and CMOS imaging chip for imaging based DNA arrays. Fluorescent dye labeled alkaline phosphatase (AP) was used as a signal generation element for the detection of DNA molecules from biological samples. Light emission from a bioluminescent enzyme reaction was transferred to fluorescent dye conjugated to the enzyme. This homogeneous BRET technique greatly improves the spatial resolution of the images, and solved the signal diffusion problem observed in DNA chemiluminescent arrays. Moreover, by using this method, it is possible for direct measurement of fluorescent signals on an imaging chip without an external light source which is required in conventional fluorescent detection.

Keywords: Bioluminescence energy transfer, DNA array, CMOS chip,

1 INTRODUCTION

Microarray technologies are widely used in molecular biology for studying gene expression, SPN profile, protein interaction, immune detection and drug discovery. These assays, in addition to using the traditional direct and indirect labeling of a biomolecule with fluorescent dyes, have recently implemented quantum dots as new fluorescent elements. The currently commercially available DNA and protein microarrays are mostly based on fluorescent detection and they are very sensitive. This sensitivity may present problems due to the fact that many bio-agents have inherent fluorescence properties which may interfere with assays. Auto-fluorescence and photo-bleaching are the common problems in these detection methods, which occurs when the fluorescent molecule no longer responds or responds poorly when exposed to excitation light. Finally, fluorescent detection systems require an expensive

external light source and detection unit in order to detect excited optical element of targeted molecules.

Bio-luminescent assays are exquisitely sensitive and not limited by overlap between the fluorescent properties of analytes and assay components, and achieve a high signal to noise ratio. Thus, bioluminescent assays may be used in the detection of low concentration target agents *in vitro* and *in vivo* [1-3]. This technology has also been reported by a few research groups for application in CCD or CMOS chip-based detection platform [4]. The optical chip-based detection platform has been shown great potential towards the development of a highly sensitive and low cost optical biosensor. However, since bioluminescence detection is based on an enzymatic reaction, the diffusion of reaction products, of which the light signal is generated, limits its applications in high density platforms due to low spatial resolution. To localize light signals, a potential solution is BRET technology. BRET is a powerful tool for reporting molecular interaction events. It is based on the resonance energy transfer between energy donor, luminescence substrates, and energy acceptor, fluorescent dyes. The efficiency of energy transfer highly depends on distance between donor and acceptor. In this work a new signal element was developed to minimize the distance between donor and acceptor as a way to increase the efficiency of resonance energy transfer and solve the problem of signal diffusion in luminescence based assays. Homogeneous BRET occurs in fluorescent dye labeled alkaline phosphatase is reported.

2 MATERIALS AND METHODS

2.1 Materials

Streptavidin and alkaline phosphatase were purchased from Sigma. Two fluorescent dyes, Alexa Fluor555 (AF555) and Alexa Fluor488 (AF488) were purchased from Molecular probes. Oligonucleotides were made by Integrated DNA Technology. Bioluminescent substrates were purchased from Michigan Diagnostics.

The CMOS imaging chip test system was a gift from Micron Corporation. All other chemicals and biochemical were analytical grade. Fluorescence spectrophotometer Perkin Elmer LS-5 and Luminescence detector Beckman coulter LA-400 were used in the tests.

2.2 Methods

Homogeneous BRET signal elements were generated by conjugation of Fluorescent dye and alkaline phosphatase (AP). AP was dialyzed in PBS buffer pH 7.2. 200 µg of dialyzed enzyme (2.75 mg/ml) was used for conjugation followed by the addition of one tenth volume of 1M NaHCO₃ at pH 9.0. To the mixture 2.1 µl of Alex Fluor555 or Alex Fluor480, an amine reactive fluorescent dye, at concentration of 10mg/ml in DMSO was added and the entire mixture was mix and then incubated at room temperature for one hour. The conjugated alkaline phosphatase-AF555 was separated from the reaction mixture using a P-30 (Bio-Rad) straw column previously equilibrated with PBS at pH 7.2. The biological function of conjugate was assayed by using luminometer. The spatial resolution of the conjugate was tested as follows: 0.2µl of a 100nM solution of alkaline phosphatase-AF555 conjugate was spotted on a 1 cm diameter nitrocellulose membrane. Non-specific reactions were blocked by treating the membrane with 1% BSA in TBS at pH 8.5 for 5 minutes. The membrane was placed on a microscope slide. Fifty micro-liter of alkaline phosphatase substrate AttoGlow 540 was added to the membrane and a slide cover was placed over the membrane. The reaction image was photographed using a cooled CCD camera both with and without the chroma filter HQ560LP. The same experiment was performed with the CMOS color chip.

3 RESULTS AND DISCUSSION

Figure 1 is a schematic diagram of homogenous BRET used as signal element in the application of DNA array on an imaging chip. The spectra of the conjugate of alkaline phosphatase- AF555 was performed to prove the concept of energy transfer. The result in Figure 2 clearly indicated that energy is transferred from luminescence reaction to the fluorescent dye. The first light emission was from the enzyme reaction with a wavelength of 450nm. The second light emission was from fluorescent dye AF555 with a wavelength of 580nm. No second light emission was observed in the spectra of AP alone (data not shown). Serial conjugate dilution was tested for BRET. We noticed that the energy transfer is significant at high concentration of conjugates. It can reach 50% of total light emitted from luminescence reaction. However, at low concentration of the conjugates, energy transfer was not significant. That may result from a quick diffusion of the substrates, since the efficiency of energy transfer very

much depends on the distance between the energy donor (substrate) and the acceptor (fluorescent dye).

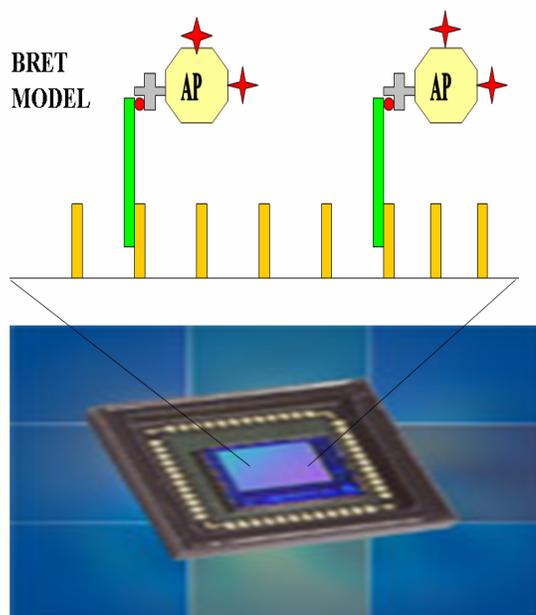


Figure 1: A detection model of BRET on a digitally controlled Bio-CMOS chip Biotin labeled target DNA molecules were capture on the chip surface. Streptavidin-AP-fluorescent dye conjugates binds to the biotin and generates BRET signal.

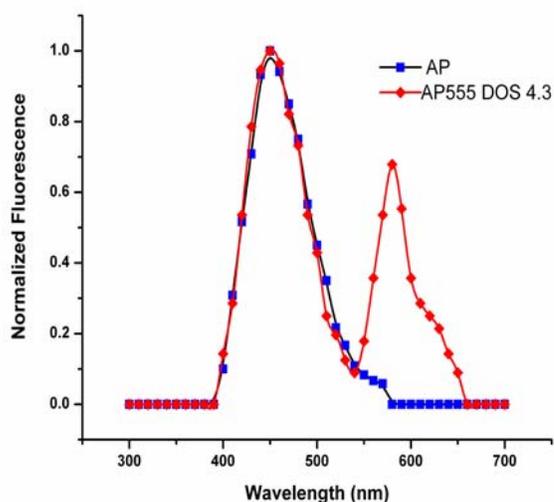


Figure 2: Comparison of fluorescence spectra of AP555 DOS 4.3 dye and AP, spectra showing and excellent BRET by AP555 DOS 4.3 dye, no BRET was observed with AP.

The comparison of energy transfer with two fluorescent dyes and three substrates (Fig 3) was demonstrated. The result showed no significant difference of BRET between two conjugates. However, there is a significant difference of BRET by using different substrates.

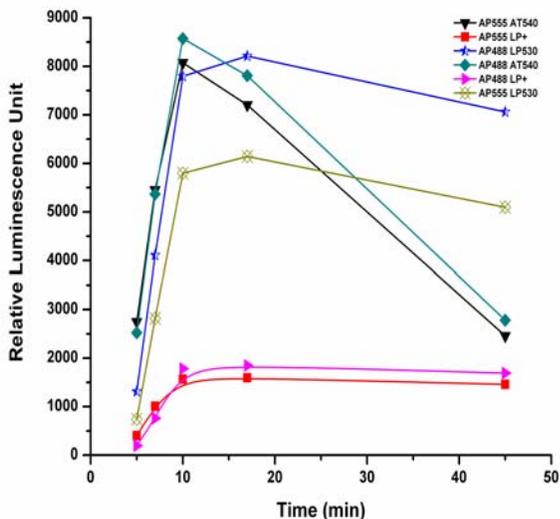


Figure 3: Comparison plot of three substrates and two fluorescent conjugates with increasing time

To determine the time and concentration related BRET, time titration experiments were performed. Serial dilutions of AP or AP-AF555 conjugates were tested to determine relative luminescence. The pattern of light emission in both AP and AP-AF555 are similar. At higher concentrations, light intensity was reduced quickly in the first three minutes after initial bursting. At low concentrations, the peak of emission was delayed, and the reduction of light intensity was relatively slow (Fig 4).

On chip detection of homogenous BRET was carried on as follows: 5ng of AP, AP conjugate and Alexa Fluor 555 were spotted on nitrocellulose membrane. Luminescence signal and BRET were detected by using substrate Attoglow 540. Figure 5 is images took by a cooled CCD camera (black & white) and a CMOS imaging chip (color). These images clearly reflect that homogeneous BRET, as a signal element, greatly improved spatial resolution of detection signals.

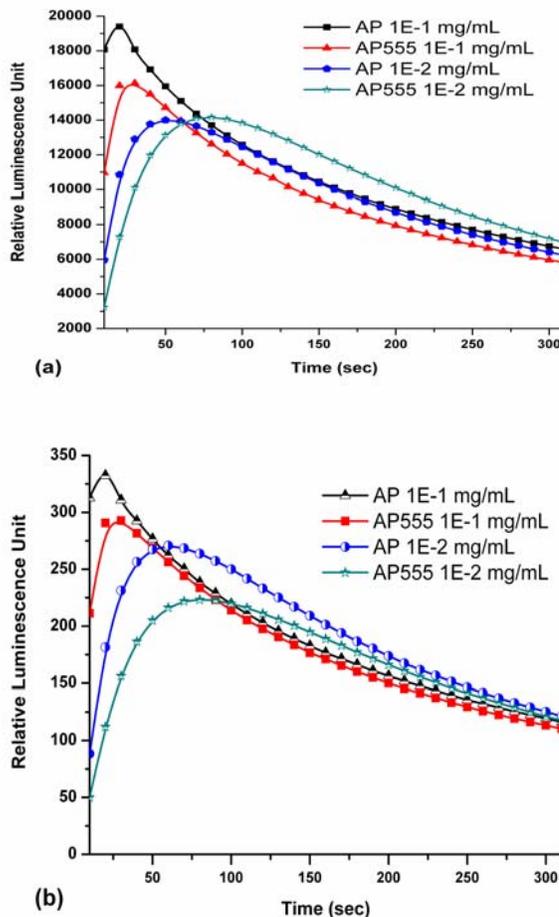


Figure 4: Concentration and time titration graph of BRET, (a) Concentration profile of AP and substrate without filter, and (b) is the same profile using 570 nm filter.

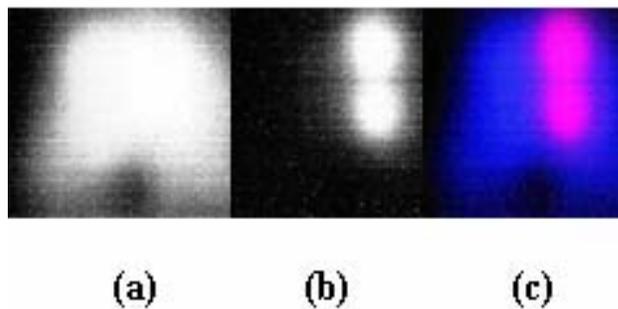


Figure 5: Cooled CCD and CMOS chip images of AP and AP-AF555. Showing two spots on the left are AP alone and two spots on the right are AP-AF555 conjugate. (a) Image was taken with no filter; (b) image was taken with a 560 nm long pass filter from Omega and (c) image was captured on a color CMOS imaging chip.

4 CONCLUSIONS

Our results have shown that homogeneous BRET as a new detection element improves spatial resolution in luminescence assays. The efficiency of energy transfer depends on the distance between energy donor and acceptor. It is also concentration and time related. At low concentrations of conjugates, energy transfer is not significant which might result from a slow degradation of substrates. That allows substrate diffusing away from conjugates, therefore decreasing the efficiency of resonance energy transfer. Significant signal of BRET was observed in the initial reaction, and a signal was decreased quickly after the initial peak. Although further investigations are required to improve the BRET efficiency, it still has a great potential to be used as a signal element in an imaging chip based bio-molecular array.

5 ACKNOWLEDGEMENTS

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