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A Fluorescent Arrayed Biosensor Using Liposome Encapsulating Calcein for Discrimination of Different Target Proteins by Principal Component Analysis

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Abstract

A bio array sensing system is newly composed utilizing different phospholipid liposomes encapsulating fluorescent molecules. We have confirmed a high output intensity of fluorescence emission due to the characteristics dependent on the concentration of fluorescent molecules when the fluorescent molecules leak from inside of the liposome through perturbed lipid membrane. After measuring a whole array image of fluorescence emission output from every element of liposome sensor by a CMOS imager system, the output of fluorescence emission from all the elements was analyzed by a statistical method of principal component analysis (PCA). It is found from obtained PCA plots that different species of proteins with several concentrations are clearly discriminated with high cumulative contribution ratio.

1. Introduction

We have proposed a preliminary detection approach by arrayed biosensor, utilizing liposome encapsulating fluorescent molecules and the corresponding time course analysis of the fluorescence [1]. As a result, it was needed firstly to make more stable and higher output intensity of fluorescence emission from a liposome element. Secondly, to investigate more different type of phospholipid for improving the sensitivity and capability of discrimination between different target biomolecules. Similar to cell membrane, cholesterol was newly incorporated into phospholipid membrane of DPPC liposome from an initial step in the liposome preparation, because it has been known that membrane fluidity changes significantly with and without the cholesterol [2,3], leading to different interaction with a target protein. Thirdly, as an essential point, it is required to analyze the arrayed data statistically. We proceed to apply PCA with a data mining tool of Weka [4].

2. Results and Discussion

Figure 1 plots fluorescence emission intensity from a liposome sensor element dependent on fluorescent calcein molecule concentration. It is known that fluorescence intensity depends on the concentration of molecule that emits fluorescence especially for calcein [5]. Simultaneously, the fluorescent calcein molecules are leaked dependent on how much the liposome membrane permits their transmission. Thus, it is important to prepare a proper calcein concentration encapsulated in the liposome for increasing and optimizing the fluorescence intensity. Here we set the calcein concentration as 100 mM because the calcein molecules are considered to leak by less than a few percent in our experiments.

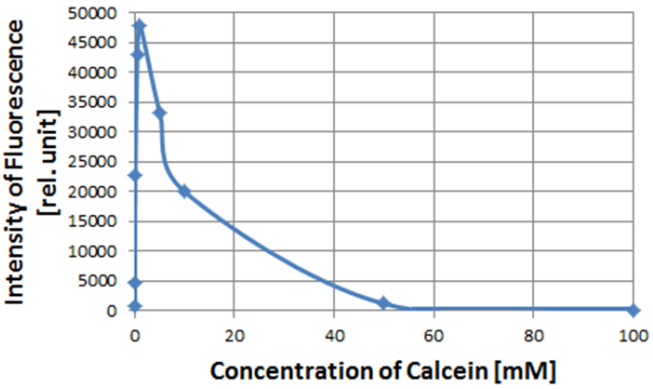

Fig. 1 Intensity of fluorescence as a function of concentration of calcein.

As illustrated in Fig. 2, a droplet of suspension (10 mM) of liposome (DPPC, DSPC, or DPPC : Cholesterol = 66 mol% : 33 mol%) encapsulating calcein solution (100 mM) was spotted in a microwell, thereafter a solution of target protein (CAB or Lysozyme) was supplied. Fig. 2 also shows the cross-sectional illustration of photometric system of fluorescence. Excitation light emitted from Blue-LED reflects by the optical filter and reaches the arrayed microwells. Caused by the interaction between liposome and protein, encapsulated calcein leaks from inside of liposomes, which are filled in the microwells. Fluorescence of the leaked calcein molecules passes through the optical filter and longpass filter, and is detected by a CMOS imager to evaluate the liposome-protein interaction. In the fabricated photometric system, fluorescent images of every element in the array were obtained (Fig. 3) and analyzed. For horizontal direction, sensor elements were filled with different species of phospholipid liposome, and for vertical

direction, they were supplied with different concentration of target protein. Differences in fluorescent intensity among the sensor elements are observed dependent on the species of phospholipid and the concentration of target protein.

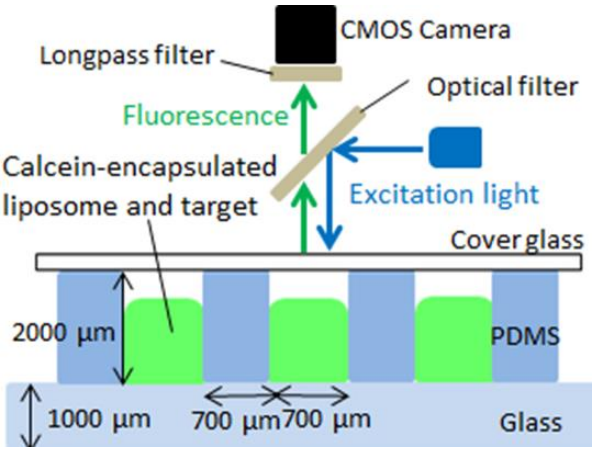

Fig. 2 A cross-sectional view of microwell chambers of array with illustration of photometric system of fluorescence.

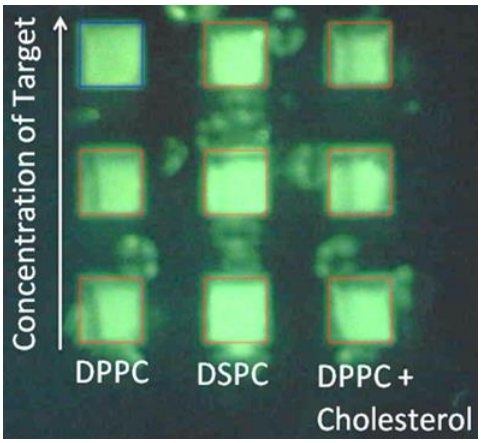

Fig. 3 An example image of a whole fluorescent liposome array sensor.

Figure 4 illustrates a result of PCA plot, where we used correlation matrix in calculation and relative fluorescence intensity defined as RF = ΔI / I_0 = (I - I_0) / I_0 (I: fluorescence intensity after 60 min from addition of target protein, I_0: fluorescence intensity just after the addition). Averaged results (N=3) are plotted for each protein with a concentration. It is found that the two target proteins with different concentrations are discriminated in the plot. We think this analyzed result is sufficiently available as cumulative contribution ratio larger than 90% are obtained for both PC1 and PC2.

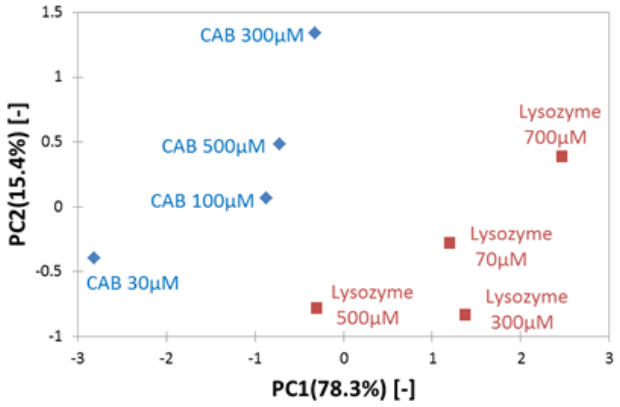

Fig. 4 Scatter plots of the two target proteins with different concentration by Principal Component Analysis.

3. Conclusions

A fluorescent array sensor utilizing different phospholipid liposome encapsulating calcein molecules was fabricated to discriminate target proteins. Fluorescence of calcein leaked from inside of liposomes caused by liposome-protein interaction was detected by a developed photometric system. Obtained relative fluorescent intensity was analyzed by principal component analysis. It is clear from the PCA plot that the species and concentration of target proteins (CAB and Lysozyme) can be successfully discriminated.

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