Application Note

FP-0022

Sensitivity evaluation of biosensor by using FRET

Introduction

FRET is the mechanism that the energy is transferred non-radiatively from an excited state donor to a neighbor acceptor (Figure 1). FRET is an abbreviation for Forester (or fluorescence) resonance energy transfer.

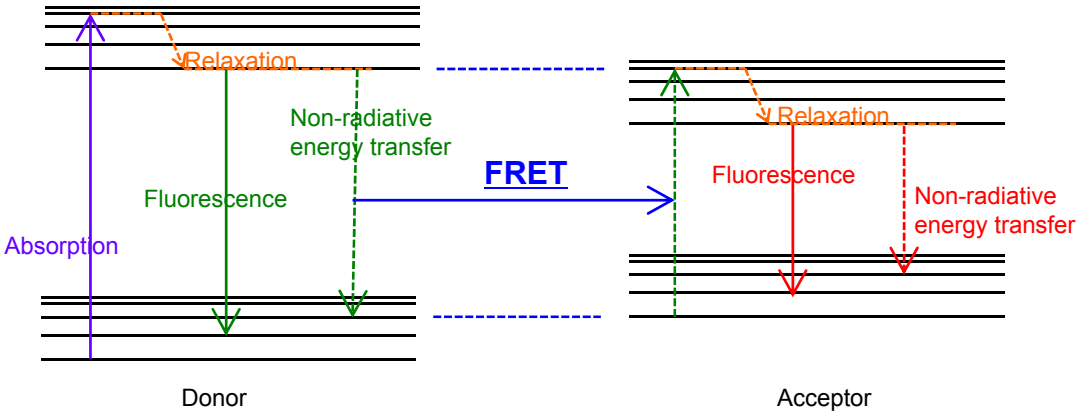


Fig.1 Energy transition in a FRET pair

FRET is monitored by the spectrofluorometer, which measures the fluorescence/quenching of acceptor or excited donor. FRET efficiency depends on the following factors (Figure 2).

1. Spectral overlap between the donor and acceptor

As the overlap area of the donor fluorescence spectrum and the acceptor absorption spectrum is larger, FRET efficiency is higher.

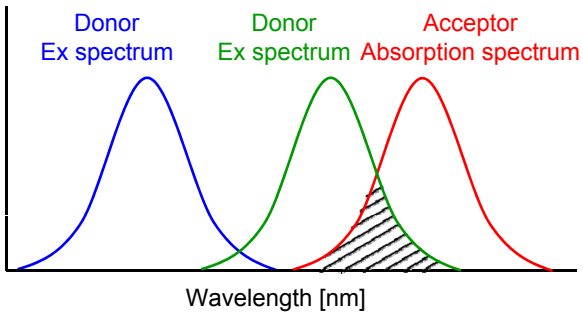
2. Distance between donor and acceptor

FRET efficiency is inversely proportional to the six power of distance between the donor and acceptor.

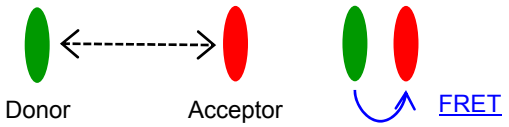
3. Orientation of donor and acceptor

FRET efficiency is a maximum when the two dipole moments are parallel or anti-parallel to each other, and no energy transfer occurs when the dipole moments are perpendicular to each other.

1. Spectral overlap between the donor and acceptor



2. Distance between donor and acceptor



3. Alignment of donor and acceptor

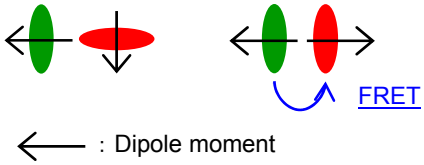


Fig.2 Factors that influence FRET

JASCO INTERNATIONAL CO., LTD
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Typically, when the distance between the donor and the acceptor is 1 -10 nm, FRET is occurred. This mechanism is used for the following applications.
- Monitoring the protein conformational change by fusing the donor and acceptor to two different sites in the same protein (intramolecular FRET [1])
- Monitoring the protein-protein interactions by fusing the donor and acceptor to two different host proteins (intermolecular FRET [1])

One of FRET application examples is the murine anti-TNT monoclonal antibody (anti-TNT mAb), which can form a complex with trinitrotoluene (TNT). Anti-TNT mAb absorbs the excitation light of 280 nm, and produces the fluorescence light of 340 nm. When anti-TNT mAb forms a complex with TNT, FRET is occurred between the anti-TNT mAb (donor) and TNT (acceptor). The energy is transferred from the anti-TNT mAb excited at 280 nm to the TNT, and the fluorescence at 340 nm from the anti-TNT mAb is quenched (Figure 3). By utilizing this interaction as biosensor, this application note shows the evaluation of the sensitivity between the aromatic nitro compounds and anti-TNT mAb [2].

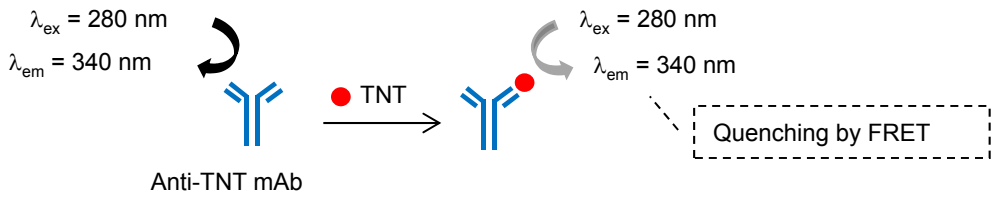

Fig.3 Schematic illustration of fluorescence quenching by FRET

Keyword
FRET, Antigen-antibody reaction, Explosive detection

Measurement
10 uL of 4.4x10-8 mol/L TNT solution was added to 800 uL of 1.6x10-7 mol/L anti-TNT mAb, and the fluoresence measurement was performed. After measurement, the final concentration of TNT was increased step-by-step by adding each 10 uL of TNT solution (4.4x10-7 mol/L, 4.4x10-6 mol/L, 4.4x10-5.5 mol/L and 4.4x10-5 mol/L), and fluorescence measurement of each different TNT concentration solution was performed.
Regarding the other aromatic nitro compounds, similar measurements were performed.

Reagents and Samples
murine anti-TNT monoclonal antibody (anti-TNT mAb): 1.6x10-7 mol/L [1]
Aromatic nitro compounds
Trinitrotoluene (TNT) : 4.4x10-8, 4.4x10-7, 4.4x10-6.5, 4.4x10-6, 4.4x10-5.5, 4.4x10-5 mol/L
Trinitrobenzene (TNB) : 4.7x10-8, 4.7x10-7, 4.7x10-6.5, 4.7x10-6 mol/L
2,6-dinitrotoluene (2,6-DNT) : 5.5x10-8, 5.5x10-7, 5.5x10-6, 5.5x10-5, 5.5x10-4 mol/L
2-Nitrotoluene (NT) : 7.3x10-8, 7.3x10-7, 7.3x10-6, 7.3x10-5, 7.3x10-4 mol/L
Hexahydro-1,3,5-trinitro-1,3,5-triazine (RDX): 4.5x10-8, 4.5x10-7, 4.5x10-6, 4.5x10-5, 4.5x10-4 mol/L
Solvent
pH 7.4 phosphate buffer

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Measurement condition

Ex wavelength: 280 nm	Measurement range:	300 - 500 nm	
Excitation bandwidth:	1 nm	Emission bandwidth:	20 nm
Scan speed:	200 nm/min	Data acquisition interval:	0.5 nm
Response:	1 sec	Sensitivity:	500 V
Spectral correction:	ON		

Measurement results

The fluorescence spectra of mixture solution of anti-TNT mAb and TNT were shown in Figure 4. As the TNT concentration increases, the fluorescence intensity of anti-TNT mAb becomes weak. Similar measurements were performed using other aromatic nitro compounds as samples. The degree of progress of complex formation reaction was determined by the decrease of the fluorescence intensity at 340 nm.

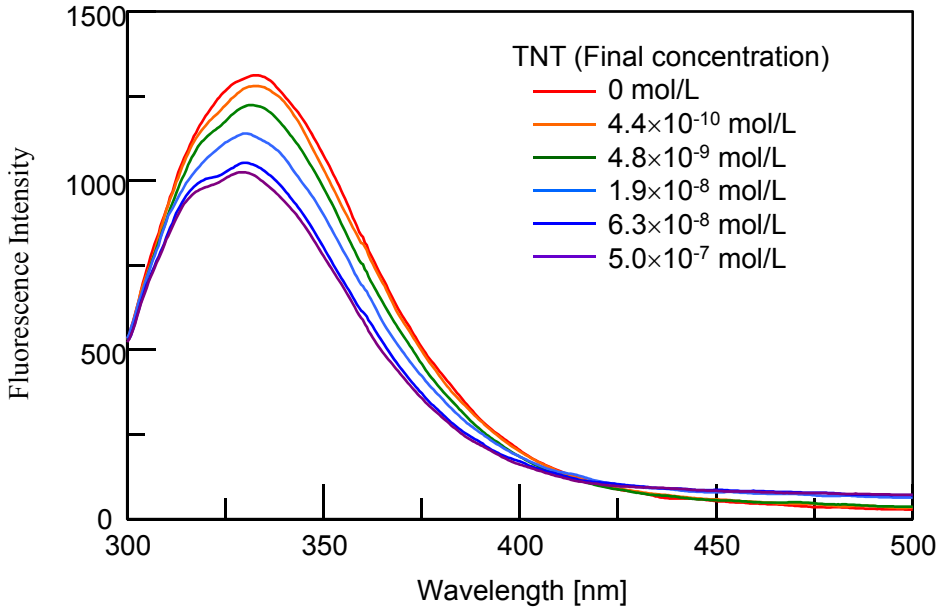

Fig.4 Fluorescence spectra of solutions containing anti-TNT mAb and various concentration of TNT

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Figure 5 shows the relations between the dose of aromatic nitro compounds and the reaction ratio. From this curve, the half maximal inhibitory concentration (IC50) was estimated (Table 1). This result shows that anti-TNT mAb have sensitivity to TNT, TNB, NT and 2,6-DNT, especially have highly sensitivity to TNT and TNB whereas can't detect RDX.
From these results, anti-TNT mAb is expected as high sensitive nitro compounds biosensor without using fluorescent label.

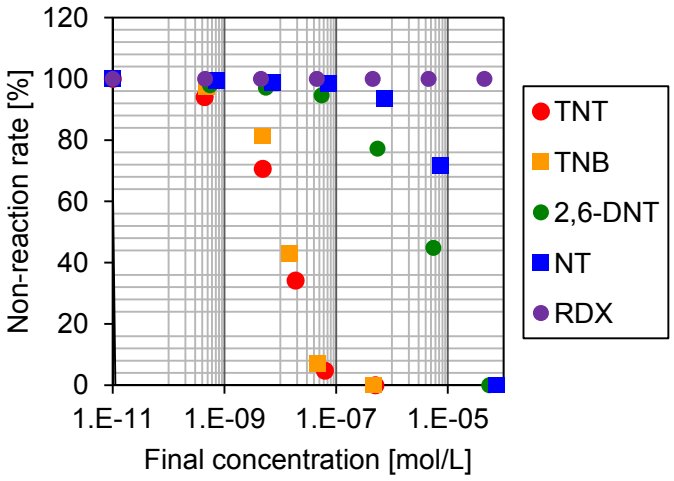


Fig.5 Dose-response curve of Anti-TNT mAb and aromatic nitro compounds

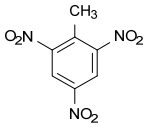
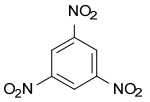
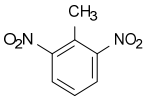
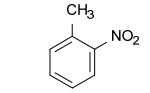
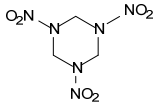
Name	Structure	IC_{50} [M]
TNT		4.0E-08
TNB		3.8E-08
2,6-DNT		7.9E-06
NT		3.6E-04
RDX		-

Table 1 Analysis of binding of different explosive compounds to anti-TNT

Acknowledgement
Special thanks to Hiroshi Nakayama of Panasonic corporation who cooperated with the experiment.

[1] Kevin Truong and Mitsuhiko Ikura, Current Opinion in Structural Biology 2001, 11:573–578
[2] Satoko Suzuki, Toshifumi Uchiyama, Ken-ichi Akao, Koushi Nagamori, Hiroshi Nakayama and Yuji Ito, TNT sensing using non-labeling FRET, Japan-Taiwan Medical Spectroscopy International Symposium/14th Annual Meeting of the Japan Association of Medical Spectroscopy, AWAJI, JAPAN 2016

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