Development of Ultra-sensitive and Super-rapid Biosensors

Platform Development

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. Versatile Platform for Detection of Antibiotics, Lipids, Small Molecules & Drug Screening .

Proteins and enzymes are created by nature to recognize specifically their partners of interacting molecules. They are thus excellent materials for constructing tailor-made biosensors if connected to a signal generating part. By studying their crystal structures and amino acid sequences from protein data banks, we use rational design approach to guide the selection of suitable amino acid residues to be replaced by other residues. This innovative approach allows the construction of a mutant with a unique cysteine residue near the ligand or substrate binding site to be labeled with an environment-sensitive fluorophore for the purpose of bio-sensing. So far, using this approach and in collaboration with others, our group has successfully constructed a variety of novel and patentable fluorescent biosensors for detecting beta-lactam antibiotics from milk, arginine (amino acid) from blood, and vitamin A from blood.

We are also interested in elucidating bio-sensing mechanisms of our different fluorescent biosensors. We have proposed and are testing the hypothesis that a tailor-made fluorescent biosensor can be constructed by rational approach to sense a specific molecule which is a ligand or substrate for a particular protein or enzyme. This approach allows the development of a universal protein-based biosensor construction method to detect a large variety of important small molecules in the body.

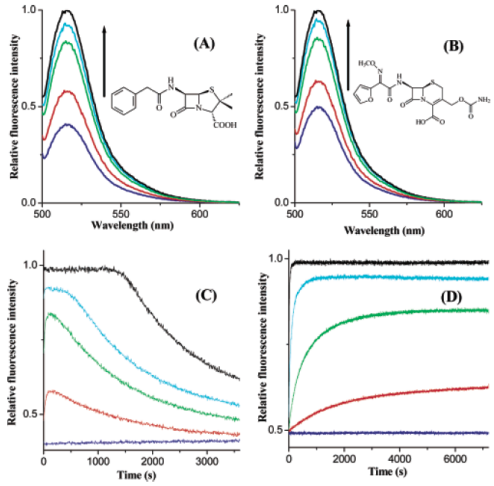


Figure 1. Fluorescence spectra of E166Cf in the presence of penicillin G (A) and cefuroxime (B) in 50 mM phosphate buffer (pH 7.0). The corresponding time-resolved fluorescence changes in the presence of penicillin G and cefuroxime as monitored at 515 nm are shown in panels C and D, respectively. Navy: 0.12 μM E166Cf enzyme. Red, green, light blue, and black, after addition of 0.1, 1.0, 10, 100 μM antibiotics respectively.

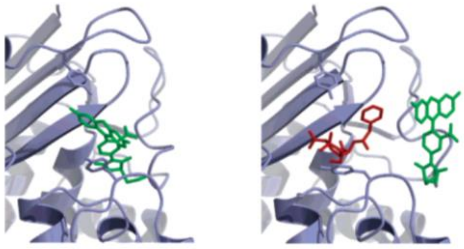
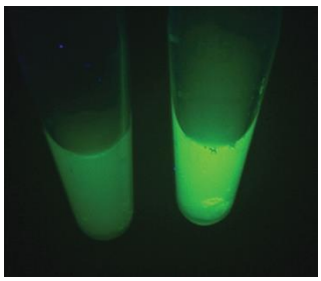


Figure 2. Molecular models of the fluorescein label (green) on E166Cf before (left) and after (right) binding with penicillin G (red).



Fluorescein-labelled biosensor in water – the one on the right shows a prominent increase in fluorescence intensity with antibiotics added.

Representative Publications

1. Man-Wah Tsang; Pak-Ho Chan; Pui-Kin So; Dik-Lung Ma; Chun-Wai Tsang; Kwok-Yin Wong; and *Yun-Chung Leung. Engineered Amp C β-Lactamase as a Fluorescent Screening Tool for Class C β-Lactamase Inhibitors. ANALYTICAL CHEMISTRY, v. 83, (6), 2011, Mar, p. 1996-2004
2. Chan PH, So PK, Ma DL, Zhao Y, Lai TS, Chung WH, Chan KC, Yiu KF, Chan HW, Siu FM, Tsang CW, **Leung YC*** and Wong KY* (2008) Fluorophore-labeled beta-lactamase as a biosensor for beta-lactam antibiotics: a study of the biosensing process. Journal of the American Chemical Society 130, 6351-6361.

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