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FLUORESCENCE ENHANCERS FOR FLUOROPHORE MEDIATED BIOSENSORS FOR CARDIOVASCULAR DISEASE DIAGNOSIS

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1. INTRODUCTION

When heart muscles are damaged due to lack of oxygen, biomolecules, such as myoglobin (MG), C-reactive protein (CRP), cardiac troponin I (cTnI), and B-type natriuretic peptide (BNP), are released into the circulation system.[1-4] Clinically significant ranges of these biomarkers blood plasma are: 4.1 ~ 41 nM for MG[1]; 5.6 ~ 56 nM for CRP[2,3]; 31 ~ 310 pM for cTnI[3]; and 26 ~ 260 pM for BNP.[3,4] A fiber-optic, fluorophore-mediated, multi-analyte immuno-biosensing system has been under development for rapid, accurate, and simultaneous monitoring of these biomarkers in blood. The target sensing ranges of MG and CRP are sufficient for the fiber optic biosensor. The ranges for BNP and cTnI, however, are too low for regular sensing methods, requiring an additional mechanism of increasing sensitivity.

A plasmon rich *nanogold particle* (NGP), when located at an appropriate distance from a fluorophore, can reroute the electrons that contribute to self-quenching and, therefore, enhance the fluorescence (Fig. 1a).[5] This enhancement effect is highly dependent on the distance between a fluorophore and an NGP. If it is too close from a fluorophore, an NGP quenches the entire fluorescence (Fig. 1b), and if too far, an NGP will not affect the fluorescence (Fig. 1c). It was found that the size of an NGP also affects the fluorescence enhancement, according to our study results.

We have also found a few biocompatible solvents significantly enhancing fluorescence. This enhancement is possibly caused by the shift of excitation/emission spectrums for the fluorophore[6], and/or by isomerization of the fluorophore[7], or by more fluorescence retrieval when the fluorophore-tagged, sandwich protein complex shrinks in the solvent and gets closer to the sensor surface. This study investigates the effects of the NGP and the solvent on the performance improvement of the cTnI and BNP sensors.

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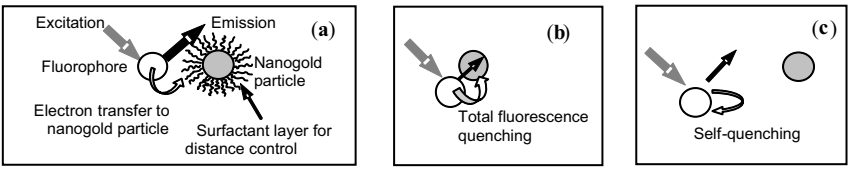

Figure 1. The effect of the distance between a fluorophore and a nanogold particle on the emission fluorescence. (**a**) At an appropriate distance: a fluorophore receives the excitation light and an NGP receives the electrons used for self-quenching, resulting in more emission light; (**b**) Too close: a fluorophore receives excitation light but the emission light is quenched, because the NGP attracts most of the electrons including the ones for fluorescence generation; (**c**) Too far: the plasmon field around the NGP does not reach the fluorophore.

2. MATERIALS, INSTRUMENTS AND METHODS

2.1 Materials and Instruments

The fluorometer (Analyte 2000) and the quartz fibers used for sensors were purchased from Research International (Monroe, WA). Protein C (PC) and PC specific monoclonal antibodies were donated by the American Red Cross (Rockville, MD). BNP (Bachem; Torrance, CA), anti-BNP monoclonal antibodies (Strategic Biosolutions; Newark, DE), cTnI, and anti-cTnI monoclonal antibodies (Fitzgerald; Concord, MA) were used to prepare BNP or cTnI sensors. Nanogold particles (2, 5 and 10 nm) linked with tannic acid (3 nm) were obtained from Ted Pella (Redding, CA). L-glutathion (1 nm) and 16-mercaptohexadecanoic acid (2 nm) used for *self-assembled monolayer* (SAM) formation on the NGP were from Sigma/Aldrich (St. Louis, MO). The fluorophores, Fluorolink™ Cyanine 5 (Cy5; 649/670 nm for excitation/emission) and Alexa Fluor® 647 (AF647; 649/666 nm for excitation/emission), were purchased from Amersham Pharmacia Biotech (Uppsala, Sweden) and Molecular Probes (Eugene, OR), respectively.

2.2 Methods

All sensors and sensing samples were prepared following the protocols established by Kang, *et al.*[8-10] As a means to adjust the distance between a fluorophore and an NGP, SAMs at particular thicknesses were immobilized onto the NGP surface. The resulting NGPs linked with SAMs (NGP-SAMs) were purified using a DispoDialyzer® dialysis tube (Spectrum Laboratories; Rancho Dominguez, CA) before use.

The protocols for BNP and cTnI biosensing were based on the PC sensing, described by Spiker and Kang.[8] When the enhancers (NGP-SAMs, solvent, or the mixture) were used, two more steps were added to the original protocol: 1) The new baseline with the enhancer, and 2) applying the enhancer after the washing step following the surface immuno-reactions between the analyte and the fluorophore-linked second antibody.