# Chapter 30
# Highly Sensitive Rapid, Reliable, and Automatic Cardiovascular Disease Diagnosis with Nanoparticle Fluorescence Enhancer and Mems

**Bin Hong[1], Junhai Kai[2], Yongjie Ren[1], Jungyoup Han[2], Zhiwei Zou[2], Chong H. Ahn[2], and Kyung A. Kang[1]**

**Abstract** Cardiovascular diseases (CVDs) have been the leading threat to human life. An effective way for sensitive and accurate CVD diagnosis is to measure the biochemical markers released from the damaged myocardial cells in the bloodstream. Here, a multi-analyte, fluorophore mediated, fiber-optic immuno-biosensing system is being developed to simultaneously and rapidly quantify four clinically important cardiac markers, myoglobin, C-reactive protein, cardiac troponin I, and B-type natriuretic peptide. To quantify these markers at a pico-molar level, novel nanoparticle reagents enhancing fluorescence were used and signal enhancement was obtained as high as ~230%. Micro-electro-mechanical system (MEMS) was integrated to this system to ensure a reliable and fully-automated sensing performance. A point-of-care, automatic microfluidic sensing system for four cardiac marker quantification was developed with the properties of 3 cm sensor size, 300 μL sample volume, 9-minute assay time, and an average signal-to-noise ratio of 35.

## 30.1 Introduction

Cardiovascular diseases (CVDs), especially the acute myocardial infarction (AMI; commonly known as heart attack), have been the top killers for human beings [1]. Rapid and accurate diagnosis of CVDs is, therefore, critically important to save lives. This can be realized by rapid, sensitive, and accurate quantification of cardiac markers released from injured cardiac muscles. Creatine Kinase-MB (CK-MB), myoglobin (MG), and cardiac troponin I (cTnI) are important markers for early diagnosis of a heart attack [2]. B-type natriuretic peptide (BNP) and C-reactive protein (CRP) are crucial markers for the diagnosis of congestive heart failure (CHF) and acute coronary syndromes (ACS) and also for the accurate prognosis after an AMI insult [3–4]. Our effort is

[1]Department of Chemical Engineering, University of Louisville, Louisville, KY 40292.
[2]Department of Electrical and Computer Engineering and Computer Science, University of Cincinnati, Cincinnati, OH 45221.

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focused on developing a highly sensitive, reliable, and user-friendly, point-of-care sensing device, utilizing nanoparticle reagents and micro-electro-mechanical-system (MEMS) technique.

The main challenge in developing a biosensor is the low concentrations of biomarkers in biofluid (very often only a few tens of pico-moles and less) at the early stage of disease [5]. Since our sensing is interrogated by fluorescence, fluorescence enhancement can improve the sensitivity. Nanogold particles (NGPs), possessing strong plasmon polariton fields on the surface, can reroute lone-pair electrons (normally contributing self-quenching) of a fluorophore to NGPs, resulting in fluorescence enhancement [6–7]. Some biocompatible solvents were also found to enhance fluorescence, by shifting the fluorophore excitation/emission wavelengths and/or increasing the number of *trans* carbon double bonds [6–7]. To maximize the enhancement effect, NGPs and solvents were combined, forming nanogold particle reagents (NGPRs). According to our previous results [7], the mixture of 5 nm sized NGPs coated with 2-nm thick self-assembled monolayer (5nmNGP-SAM2nm) in 1-butanol has shown to be an excellent enhancer.

MEMS technique improves the performance of biosensors by providing micro-fabrication tools, the consistency in operation, and compactness, as well as mass production capability. For a reliable and fully automated sensing performance with a minimal system size, MEMs was integrated to our sensing system.

In this paper, a sensitive and accurate cardiac marker sensing system with the application of NGPR and MEMS is reported. With this system, simultaneous four-cardiac marker quantification was completed in 9 minutes at an average signal-to-noise (S/N) of 35.

## 30.2 Materials, Instruments, and Methods

### *30.2.1 NGP, Solvent and NGPR-Related Study*

The 5 nm nanogold particles coated with tannic acid (Ted Pella, Redding, CA) and 16-mercaptohexadecanoic acid (MHA; Sigma/Aldrich, St. Louis, MO) were used to synthesize 5nmNGP-SAM2nm by self-assembling MHA on the NGP surface [6]. For the butanol based NGPR, 5nmNGP-SAM2nm was then dispersed in pure 1-butanol (Sigma/Aldrich).

### *30.2.2 Cardiac Marker Sensors and Assay Protocol*

Human BNP was purchased from Bachem (Torrance, CA). Monoclonal IgG against human BNP, was from Strategic Biosolutions (Newark, DE). Human cTnI, MG, and CRP, and their respective monoclonal antibodies were obtained from Fitzgerald Industries (Concord, MA). Plasma samples with cardiac

markers were prepared by adding a known amount of cardiac markers to the emulated human plasma. The emulated plasma is 103 mg/ml human serum albumin (HSA; Sigma/Aldrich) in the PBS buffered solution [8]. The fluorophore, Alexa Fluor® 647 (AF647; max. excitation/emission wavelengths, 649/666 nm), was from Invitrogen (Carlsbad, CA). Four cardiac marker biosensors were constructed, following the protocol established by Tang et al [5]. The fluorometer with four sensing channels (Analyte 2000™) was from Research International (Monroe, WA). Briefly, the monoclonal antibody (1°Mab) against the respective marker is immobilized on the optical fiber surface *via* streptavidin-biotin bond and the sensor is encased in a chamber. During the assay, the sample is injected to the sensing chamber. The target marker binds specifically to the 1°Mab on the sensor surface. After the sample incubation, unbound molecules are washed away from the sensing chamber. Next, the fluorophore tagged, second monoclonal antibody (fluorophore-2°Mab) is applied to the sensor. When the surface immobilized fluorophores are excited by the laser light, the emitted fluorescence is detected by the fluorometer. For the sensing with NGPR, NGPR is applied before the sample incubation for the baseline [6]. NGPR is also applied after the incubation of fluorophore-2°Mab and sensor washing. The fluorescence signal difference between the baseline and after the sandwich complex formation is correlated to the analyte concentration in the sample. Here, the enhancement is defined as the increase in the fluorescence signals by using NGPR divided by the fluorescence from same sample without using NGPR (control).

### *30.2.3 Microfluidic Sensing System Utilizing MEMs*

To generate micro-turbulence inside the sensing chamber, bumps (or baffles) were added on the upper and bottom sides of the microchamber (i.e., serpentine microchannel). The sensing module with the serpentine microchannels as well as the microchannel network were microfabricated as described by Sohn, et al [9]. The computer software LabVIEW™ (version 7.1) and a data acquisition card DAQ (USB-6008, 8 inputs, 12 bits, 10 ks/s, multifunctional I/O, National Instruments; Austin, TX) were used to control all electronic parts in the flow control unit. Electronically controllable micro-solenoid pump (12 v, 50 μL per stroke, 2 W) and 7 micro-solenoid valves (12 v, 280 mW, Lee Co.; Westbrook, CT) were for the automatic flow control. A drive circuit with a power plug, a power switch, and a power LED were customized by our research group.

## 30.3 Results and Discussion

Our fluorophore mediated, fiber-optic immuno-sensor is a highly sensitive detection tool and, therefore, it can be used for various human disease diagnosis/prognosis [5,8,10]. In our study for the quantification of BNP in plasma (without

using any enhancers), the sensitivity of our system was found to be two orders of magnitude higher than that of enzyme-linked immunosorbent assay (ELISA) (data not shown). However, for rapid cardiac marker quantification, especially for BNP and cTnI, due to their extremely low concentrations in plasma at an early disease stage, additional sensitivity improvement was needed.

### *30.3.1 Cardiac Marker Sensing Using NGPR*

As previously stated, 5nmNGP-SAM2nm in 1-butanol was found to be an excellent fluorescence enhancer. Its enhancement effect was, therefore, tested for a 3-cm BNP sensor. Figure 30.1a shows the sensing performance of BNP sensor with and without the NGPR. With the NGPR, the signal intensity was found to be 410% greater than that without NGPR. This NGPR was also tested with four cardiac marker sensors encased in a four-microchannel sensing module (Fig. 30.1b). The sample was the mixture of four cardiac markers in the emulated human plasma. The concentration of each marker was selected to be at its lower limit in the sensing range, because this is the condition requiring the enhancement the most. Results showed that NGPR is able to increase the signal intensities of BNP, cTnI, MG, and CRP sensors by 60, 50, 180, and 230%, respectively. In general, the signals from the sensors for the markers with higher concentration ranges (MG, 4–40 nM; CRP, 5.6–56 nM) were enhanced more than those for the sensors with lower concentration markers (BNP, 26–260 pM;

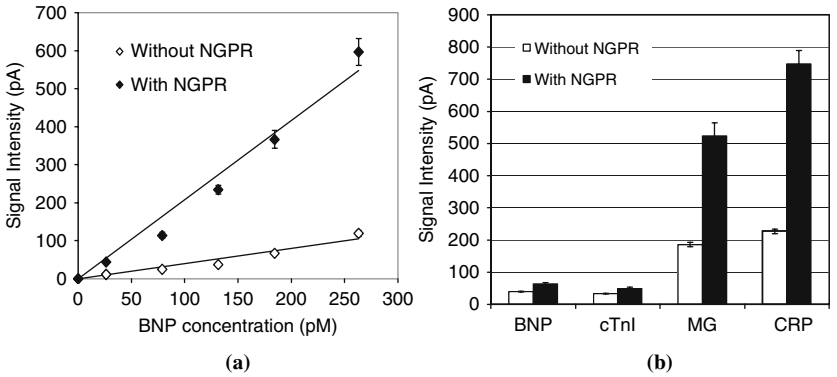


**Fig. 30.1** Sensing performances with and without NGPR: (a) BNP sensor in the BNP sensing range and (b) four cardiac marker sensors for their lower sensing limit in the microfluidic sensing system. [Experimental conditions: For (a), 3-cm sensor; 3/4 minutes for the sample and AF647-2°Mab incubation; flow velocity at 1.2 cm/sec, NGPR, 5nmNGP-SAM2nm in 1-butanol, capillary microchannel, automatic sensing. For (b), cardiac markers at their lower limits; mixture of AF647-2°Mab; serpentine sensing module, other operation conditions were the same as (a).]

cTnI, 31–310 pM). The reason for different enhancement levels should be studied further.

### *30.3.2 Cardiac Marker Sensing Chamber with Microfabricated Serpentine Structure*

For biosensors utilizing surface reaction, effective analyte mass transport from bulk media to sensor surface is important for a rapid assay. Convective application of liquid samples/reagents to the sensor surface was proven to improve the sensitivity [10]. However, reasonable flow rates without damaging the microchannels of the sensing system are in a laminar flow range and limit the analyte transport, especially for the sample with a very low analyte concentration [5]. Well-designed microchannels that can create local turbulence facilitate the analyte transport to the surface better [8]. For this purpose, a series of bumps/baffles were microfabricated on the inner surface of the microchannel (serpentine microchannel, Fig. 30.2a and b). Out of various bump configurations that we have tested, the half-circular bump series, with the dimensions of 1200 μm diameter, 400 μm height, and 1200 μm spacing between two adjacent bumps, were found to be very effective [8]. The sensing performance of 3-cm BNP and MG sensors was studied for the effectiveness of this serpentine microchannel and the results were compared with those from the channels without bumps (capillary microchannel; I.D. = 1400 μm). These two molecules were selected for the test because BNP has a low analyte sensing range (26∼260 pM) and MG, a high range (4,000∼40,000 pM). For BNP sensing (Fig. 30.2c), serpentine microchannel presented approximately 30∼90% higher signals than the capillary microchannel. For MG sensor, only a slight signal increase (0∼6%) was exhibited (Fig. 30.2d), probably because, due to its high sensing range, MG is not mass transport limited.

### *30.3.3 Sensing Operation Utilizing MEMS*

Automation of sensing system operation is important for the assay consistency, reliability in operation, and user-friendliness. In the multi-cardiac marker sensing system, MEMS technique was implemented for the automatic flow control unit (Fig. 30.3ab). An electronically controllable micro-pump and seven microvalves were used to deliver the sample and reagents to the sensing module and the microchannel network. The automatic control of the micro-pump, micro-valves, fluorometer, and other electronic parts were done by a customized LabVIEW™ code with an easy and simple interface. Therefore, a MEMS based biosensing system was developed for simultaneous, quantitative measurement of the four cardiac markers (Fig. 30.3c). Using this automated sensing system, for all four

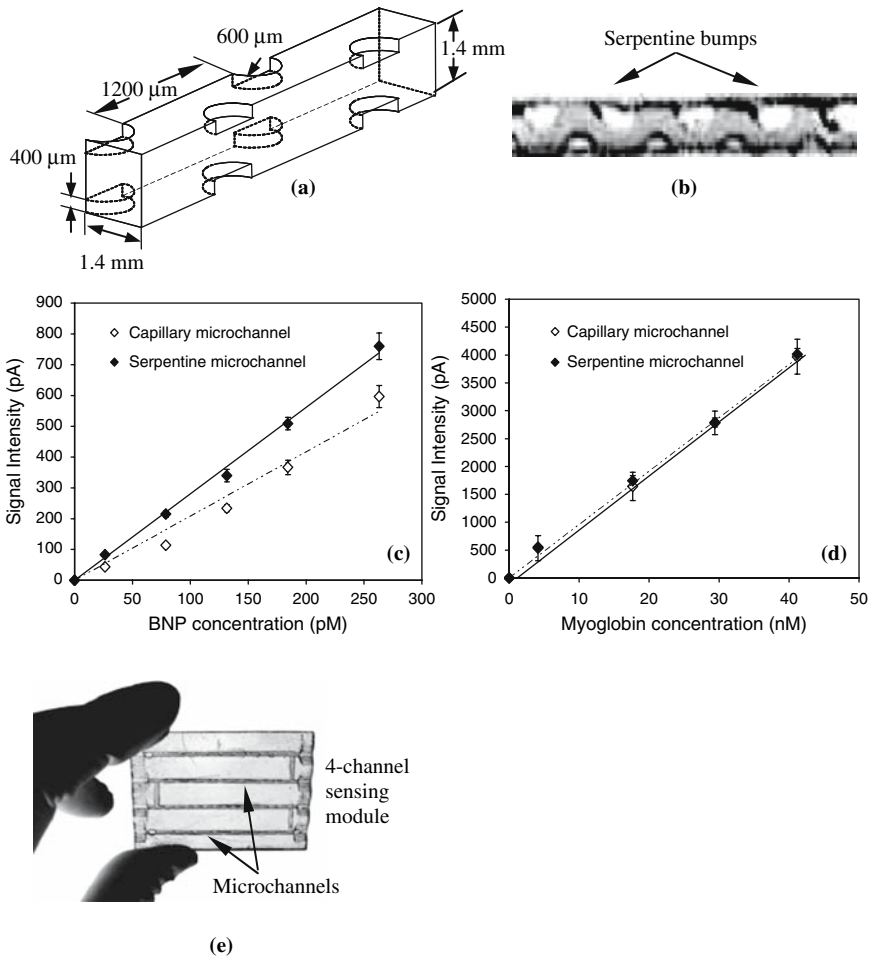


**Fig. 30.2** (a) Schematic diagram of the serpentine microchannel and (b) its actual side view. [Structure: a series of half-circular bumps at 600 μm radius, 400 μm height and 1200 μm spacing between bumps and 1.4×1.4 mm square cross-section]; the sensing performance of (c) BNP and (d) MG sensors using capillary microchannel (◇) and serpentine microchannel (◆). [Experimental conditions: 3-cm sensor, 3/4 minutes for sample and AF647-2°Mab incubation, flow velocity at 1.2 cm/sec, NGPR, 5nmNGP-SAM2nm in 1-butanol , automatic sensing.]; and (e) a four-channel serpentine sensing module.

sensors, the S/N ratio in average was doubled from 18 to 35, with signal intensities similar to those by the manual operation. In addition, with the micro-fabricated flow network, the sample volume required for each assay decreased from 1 mL to 300 μL.

In order for our sensing system to be used for a rapid diagnosis of diseases, especially for AMI, a shorter assay time is highly desired. Here, the sensing performance of four cardiac marker sensors in our serpentine sensing module

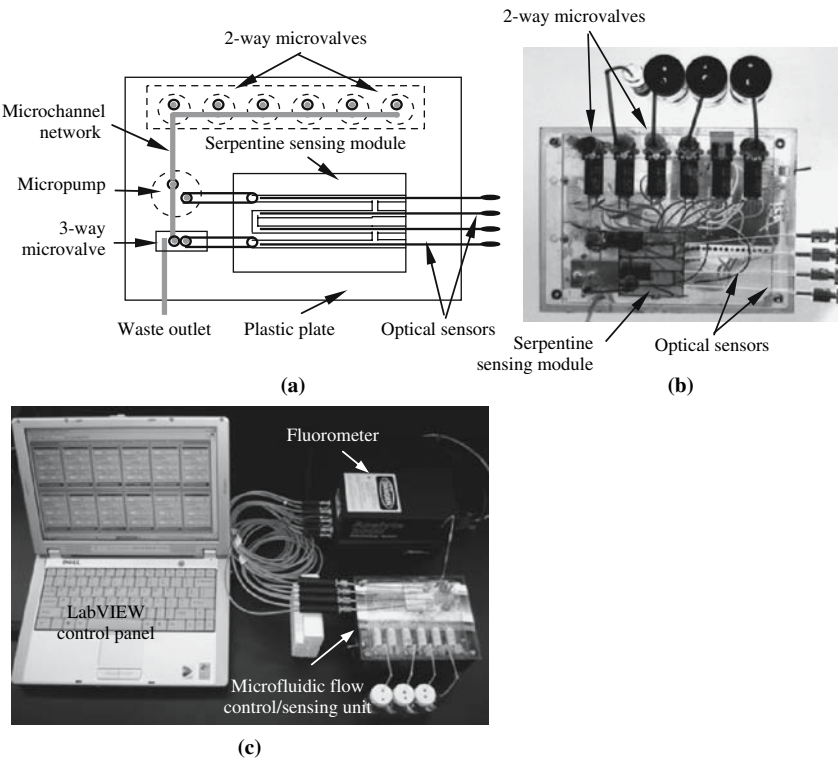


**Fig. 30.3** Automatic sensing system: (a) Schematic diagram and (b) the top view of the actual microfluidic sensing unit: imbedded microchannel network, micro-pump, micro-valves, serpentine sensing module; (c) The entire sensing system including the laptop computer with LabVIEW control panel, microfluidic flow control/sensing unit with serpentine sensing module, fluorometer with four detection channels.

(Fig. 30.2e), was studied with changes in the incubation times for the sample and the AF647-2°Mab, by the new assay protocol with NGPR. Plasma samples with four markers at their lower concentration limits (e.g., 0.1 ng/ml BNP, 0.7 ng/ml cTnI, 70 ng/ml MG, and 700 ng/ml CRP) were incubated for 1, 2 or 3 min, at a constant incubation time of 4 min for the AF647-2°Mab mixture (Fig. 30.4a). Results of BNP and cTnI sensors were shown in one figure (Fig. 30.4a1), while MG and CRP in another figure (Fig. 30.4a2), because of their similar sensing ranges. For cTnI (Fig. 30.4a1, $\leq$) and MG (Fig. 30.4a2, ()), the signal intensities increased sharply only after 2 min. From the results of four sensors, 3 min seems the optimal reaction time for the assay. Although the signals for all sensors may increase after 3 min, the signal intensities at 3 min were all high with high S/N ratios. Similarly, the effect of the AF647-2°Mab incubation time (1, 2, 3, or 4 min) on sensing performance was studied with a constant sample incubation time of 3 min. All four sensors showed a similar

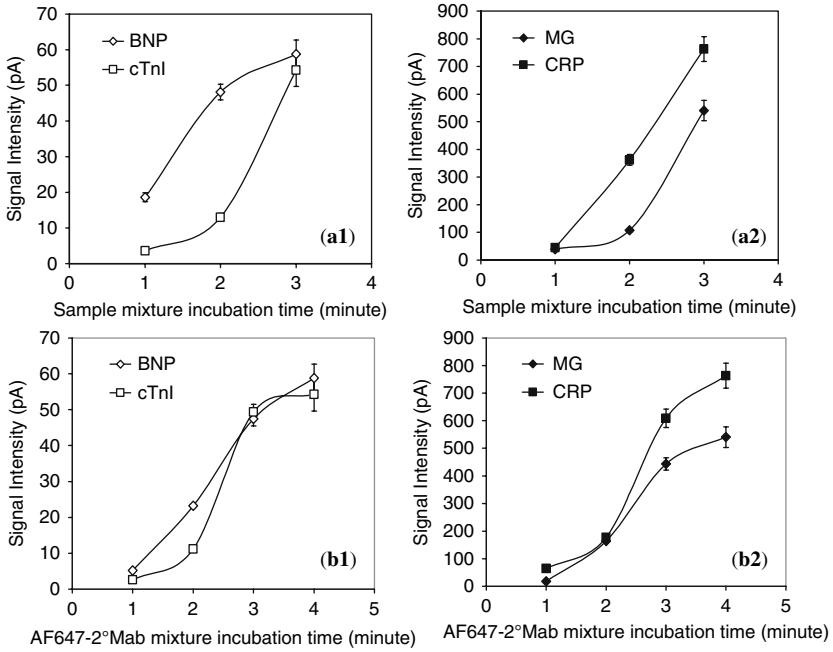


**Fig. 30.4** The effects of reaction time for (a) sample and (b) AF647-2°Mab on the sensing performance. [Experimental conditions: four cardiac markers at their lower limit of sensing ranges; sensor size, 3 cm; flow velocity, 1.2 cm/sec; NGPR, 5nmNGP-SAM2nm in 1-butanol; serpentine sensing module; automatic sensing.]

signal profile with the increase of the AF647-2°Mab incubation time (Fig. 30.4b). At 3 min, the signal increase was slowly tapered, indicating 3 min is sufficient.

In a sum, with the application of NGPR and MEMS, the four cardiac marker biosensing can be completed within 9 minutes (3, 3, and 3 min for sample incubation, AF647-2°Mab incubation, and all other times such as sample/reagent delivery, sensor washing and regeneration, respectively) with a 3-cm sensor size, and a high S/N ratio of 35.

## 30.4 Conclusions

A MEMS based, multi-analyte, point-of-care biosensing system was developed to simultaneously quantify four important cardiac markers in blood plasma. To improve the sensitivity for analytes, fluorescence enhancing NGPR was applied. The sensitivities of BNP, cTnI, MG and CRP sensors increased by 60%, 50%, 180% and 230%, respectively. A serpentine sensing chamber was microfabricated to improve the analyte mass transport and the structure improved the sensitivity well, especially for the analyte with low concentration.

MEMS technology was also incorporated to the system for a reliable detection and user-friendly operation. The sensing consistency of the system (S/N ratio) was doubled, the assay time became 9 min and the sample volume decreased to 300 μL.

Our MEMS based, multi-analyte biosensing device can be used for quantifying disease-representing multi-biomarkers, rapidly, accurately, and user-friendly.

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