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# Ultrasensitive detection of lead (II) based on fluorescent aptamer-functionalized carbon nanotubes

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ABSTRACT

Lead contamination is a serious environmental problem with toxic effects in human. Here, we developed a simple and sensitive sensing method employing ATTO 647N/aptamer-SWNT ensemble for detection of $Pb^{2+}$. This method is based on the super quenching capability of single-walled carbon nanotubes (SWNTs), high affinity of the aptamer toward $Pb^{2+}$ and different propensities of ATTO 647N-aptamer and ATTO 647N-aptamer/$Pb^{2+}$ complex for adsorption on SWNTs. In the absence of $Pb^{2+}$, the fluorescence of ATTO 647N-aptamer is efficiently quenched by SWNTs. Upon addition of $Pb^{2+}$, the aptamer binds to its target, leading to the formation of a G-quadruplex/$Pb^{2+}$ complex and does not interact with SWNTs and ATTO 647N-aptamer starts fluorescing. This sensor exhibited a high selectivity toward $Pb^{2+}$ and a limit of detection (LOD) as low as 0.42 nM was obtained. Also this sensor could be applied for detection of $Pb^{2+}$ ions in tap water and biological sample like serum with high sensitivity.



## 1. Introduction

Lead (Pb) is one of the most hazardous heavy metals contaminant in the environment with high persistency (Kaur et al., 2013; Zhang et al., 2013). Based on Environmental Protection Agency (EPA) and the international World Health Organization (WHO) regulations, maximum acceptable $Pb^{2+}$ level in drinkingwater, are 50 $\mu g L^{-1}$ and 10 $\mu g L^{-1}$, respectively (Gupta

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and Rastogi, 2008; Zhang et al., 2013). According to the Center of Disease Control and Prevention (CDCP), the maximum acceptable level of lead in blood is 10 μg/dL (Center of Disease Control and Prevention, 1991; Gilbert and Weiss, 2006). Lead is mainly absorbed via the gastrointestinal and respiratory systems (Abdel Moneim et al., 2011) and could induce toxic effects in liver, brain, kidneys and reproductive system (Abdel Moneim et al., 2011; Reshma Anjum et al., 2011).

Sensitive and fast techniques for detection of $Pb^{2+}$ in water are in great demand. Current methods for detection of lead ions are inductively coupled plasma atomic emission spectroscopy (ICP-AES), ICP-mass spectrometry (ICP-MS) and graphite furnace atomic absorption spectrometry (GFAAS) (Aydin and Soylak, 2010; He et al., 2008; Kuang et al., 2013). These techniques are time-consuming, labor-intensive and require expensive instruments (Kuang et al., 2013).

Aptamers are among the most general sensing platforms (Ouyang et al., 2011). Aptamers are short single-stranded oligonucleotides derived from random sequence nucleic acid libraries, generated for specific targets. Aptamers are selected through an in vitro process called SELEX (systematic evolution of ligands by exponential enrichment) (Stead et al., 2010; Zhang and Zhang, 2012). Aptamers selectively bind to various targets ranging from small molecules to whole cells (Davis et al., 1998; Stoltenburg et al., 2007). Aptamers have distinct advantages over antibodies, including ease of production, small size, easy modification, stability in heat and low cost (Li et al., 2012; Medley et al., 2011; Taghdisi et al., 2011). In addition, aptamers have high affinity and selectivity and no or low immunogenicity and toxicity (Medley et al., 2011; Taghdisi et al., 2011). Because of these unique characteristics, aptamers have been broadly applied for construction of biosensors (Li et al., 2012; Zhu et al., 2011).

Among the various signal transduction protocols, fluorescence is widely used for design of aptamer-based detections, because of its high sensitivity, simplicity and easy application (Kim et al., 2011; Ouyang et al., 2011; Zheng et al., 2012). Organic dye molecules are commonly used as both the fluorophore and the quencher in sensors based on fluorescence resonance energy transfer (FRET) (Li et al., 2013b). However, organic dye molecules suffer from photo-bleaching, low quantum yield and limitations of sensitivity when applied with biological samples (Li et al., 2013a; Zhen et al., 2010). Using ATTO 647N as a fluorescent dye could surpass these problems (Kolmakov et al., 2010a; Lesoine et al., 2012).

Low efficiency of FRET between the conventional fluorophore and quencher causes a high background signal and decreases the sensor sensitivity. FRET sensor is expensive, because it needs both fluorophore and quencher dyes. In addition, when one part of the sensor is aptamer, it makes sensor vulnerable to digestion by nuclease when used in vivo. Application of carbon nanotubes (CNTs) as quencher could prevent these problems (Zhen et al., 2010).

CNTs are molecular-scale tubes of graphitic carbon (Foldvari and Bagonluri, 2008; Taghdisi et al., 2011). CNTs are categorized structurally as multi-walled carbon nanotubes (MWNTs) and single-walled carbon nanotubes (SWNTs) (Foldvari and Bagonluri, 2008; Sgobba and Guldi, 2009). Due to their unique chemical and physical properties, CNTs have been attracting attention in various fields, such as biosensing, chemistry and drug delivery (Ouyang et al., 2011; Raffa et al., 2010; Zhu et al., 2010).

Single stranded DNA (ssDNA) binds noncovalently to SWNTs by means of π-stacking interactions between nucleotide bases and SWNT sidewalls (Ouyang et al., 2011; Zhu et al., 2010). However, when aptamer interacts with its target, the complex loses this property (Ouyang et al., 2011). SWNTs are known as excellent nonquenchers by acting as an energy acceptor (Zhen et al., 2010; Zhu et al., 2010). Moreover, SWNTs could protect aptamer from nuclease digestion and facilitate application of designed aptasensor in biological fluids (Zhen et al., 2010; Zhu et al., 2010).

Therefore, in this project a fluorescent sensor based on ATTO 647N/aptamer-SWNT ensemble was developed for $Pb^{2+}$ detection. In this sensor, ATTO 647N acts as the energy donor and SWNT acts as the energy acceptor. A ssDNA aptamer, which specifically binds to $Pb^{2+}$ (Li et al., 2013b, 2010), serves as the molecular recognition probe.

## 2. Experimental

### 2.1. Materials

The fluorescence labeled $Pb^{2+}$ aptamer, 5′-ATTO 647N-GGGTGGGTGGGTGGGT-3′ and 5′-FAM-GGGTGGGTGGGTG-GGT-3′ were purchased from Microsynth (Switzerland). $Cu(NO_3)_2$, $Pb(NO_3)_2$, $AgNO_3$, $Fe(NO_3)_3{\cdot}9H_2O$, $Ni(NO_3)_2{\cdot}6H_2O$ and $NaH_2PO_4$ were provided by Sigma–Aldrich (USA). Commercial carboxyl-functionalized SWNTs was purchased from

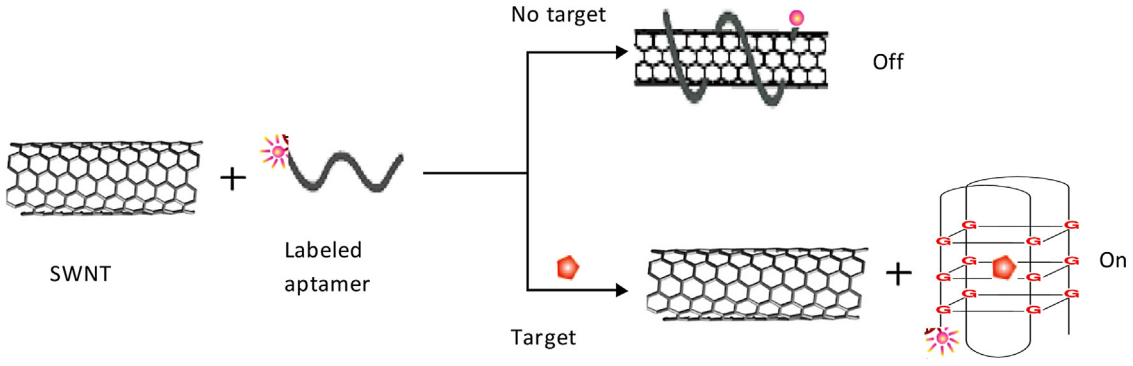


**Fig. 1 – Schematic description of $Pb^{2+}$ fluorescence assay of based on aptamer-wrapped SWNT.**