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# One-pot synthesis of quantum dot-labeled hydrophilic molecularly imprinted polymer nanoparticles for direct optosensing of folic acid in real, undiluted biological samples

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ABSTRACT

A facile and efficient one-pot approach for the synthesis of quantum dot (QD)-labeled hydrophilic molecularly imprinted polymer (MIP) nanoparticles for direct optosensing of folic acid (FA) in the undiluted bovine and porcine serums is described. Hydrophilic macromolecular chain transfer agent-mediated reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization was used to implement the molecular imprinting of FA in the presence of CdTe quantum dots (QDs). The resulting FA-imprinted polymer nanoparticles with surface-grafted hydrophilic poly(glyceryl monomethacrylate) brushes and QDs labeling not only showed outstanding specific molecular recognition toward FA in biological samples, but also exhibited good photostability, rapid binding kinetics, and obvious template binding-induced fluorescence quenching. These characteristics make them a useful fluorescent chemosensor for directly and selectively optosensing FA in the undiluted bovine and porcine serums, with its limit of detection being 0.025 μM and average recoveries ranging from 98% to 102%, even in the presence of several interfering compounds. This advanced fluorescent MIP chemosensor is highly promising for rapid quantification of FA in such applications as clinical diagnostics and food analysis.



## 1. Introduction

Molecularly imprinted polymers (MIPs) are synthetic receptors with tailor-made binding sites that are complementary in size, shape, and chemical functionality toward the target analytes. They are typically prepared by the copolymerization of a functional monomer and a crosslinker in the presence of a target molecule called “template” and the subsequent removal of the template from the resulting crosslinked polymer networks (Alexander et al., 2006; Chen et al., 2011; Haupt, 2010; Hoshino and Shea, 2011; Sellergren, 2010; Wulff and Liu, 2012; Ye and Mosbach, 2008; Zhang et al., 2006). Owing to their predetermined target selectivity, high stability, easy preparation, and low cost, MIPs have long been recognized as promising substitutes for biological receptors (e.g., antibodies and enzymes) in many applications such as separation and purification, immunoassays, chemical sensors (or chemosensors), biomimetic catalysis, or biomedicine. One of the main recent focuses in the field of molecular imprinting is to develop advanced MIP-based chemosensors because of their high potential in various rapid and selective bioanalyses (Haupt and Mosbach, 2000; Uzun and Turner, 2016; Volkert and Haes, 2014; Wackerlig and Lieberzeit, 2015; Whitcombe et al., 2011).

Recent years have witnessed rapidly increasing interest in fluorescent MIP-based chemosensors due to the easy availability of many fluorescence techniques and their high sensitivity, broad linear range, little sampling, and easy operation (Banerjee and König, 2013; Basabe-Desmonts et al., 2007; Canfarotta et al., 2013; Henry et al., 2005; Ivanova-Mitseva et al., 2012; Takeuchi et al., 2014; Ton et al., 2013; Wan et al., 2013, 2016; Zhang et al., 2014). Although significant advance has been made in the development of various fluorescent MIPs, those capable of directly optosensing small organic analytes in aqueous milieu are rather limited (Awino and Zhao, 2014; Chantada-Vázquez et al., 2016; Chao et al., 2014; Huy et al., 2014; Li et al., 2010; Liu et al., 2016; Ren and Chen, 2015; Turkewitsch et al., 1998; Wang et al., 2016; Wei et al., 2014; Wu et al., 2015; Yang et al., 2012; Zhao et al., 2012). In particular, it is still a formidable challenge to develop fluorescent MIPs that can be directly used for quantifying small organic analytes in real, undiluted biological samples due to the complex nature of such systems, which severely limits their practical applications in such areas as food safety control, environmental analysis, and clinical diagnostics (note that any dilution of biological samples will inevitably make their trace analyses more difficult or even

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impossible due to the resulting decrease in analyte concentrations). Very recently, we have reported the synthesis of organic fluorescent dye-labeled hydrophilic MIP nanoparticles for direct quantification of a small organic drug (i.e., tetracycline) in the undiluted biological samples (Niu et al., 2015). However, the well-known photobleaching problem of the organic dyes might prevent such fluorescent MIP nanoparticle-based chemosensor from being used in the continuous sensing applications (Medintz et al., 2005; Stanisavljevic et al., 2015). Since quantum dots (QDs) have proven to be fluorescent nanocrystals with many unique properties such as broad excitation spectra, narrow and tunable emission, high luminescence efficiency, and strong resistance to photobleaching (Medintz et al., 2005; Stanisavljevic et al., 2015), the development of QD-labeled MIP-based chemosensors that are capable of directly optosensing small organic analytes in real, undiluted biological samples is of high interest.

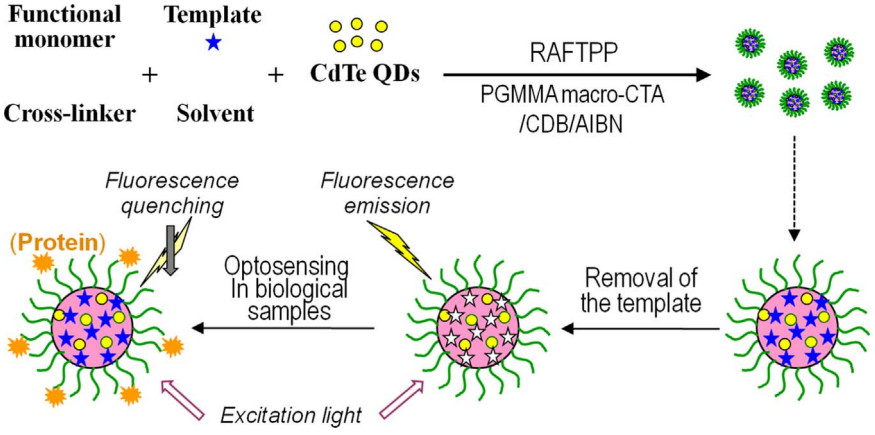
yFolic acid (FA) is a vitamin (i.e., B9) of great clinical importance to human health and its deficiency can result in many health problems with the most notable one being neural tube defects in developing embryos (Pitkin, 2007). FA has to be supplied through foods (e.g., the fortified ones containing FA) to meet our daily requirements because it can not be generated by human bodies. Therefore, the development of rapid, reliable, selective, and sensitive methods for quantifying FA in either human bodies or fortified foods is highly desirable. So far, some FA-imprinted polymers (FA-MIPs) have been designed for the selective and accurate FA detection in aqueous buffer solutions or diluted biological media (Apodaca et al., 2011; Hussain et al., 2013; Karimian et al., 2013; Pereira et al., 2014; Prasad et al., 2010), those capable of directly analyzing FA in real, undiluted biological media, however, have never been reported yet. In particular, no report on the fluorescent MIP-based chemosensor has been disclosed for optosensing FA up to now.

Herein, we report for the first time a facile and efficient one-pot approach to preparing QD-labeled hydrophilic MIP nanoparticles that are of high photostability and capable of directly optosensing FA in real, undiluted biological samples (i.e., bovine and porcine serums). MIP nanoparticles with hydrophilic polymer brushes and QD labeling were readily prepared via poly(glyceryl monomethacrylate) (briefly PGMMA) macromolecular chain transfer agent-mediated reversible addition-fragmentation chain transfer (RAFT) precipitation polymerization (RAFTPP) in the presence of CdTe QDs (Scheme 1). Their presence of hydrophilic polymer brushes and fluorescence labeling not only made them highly hydrophilic and biological sample-compatible (Ma et al., 2013; Zhang, 2013, 2014; Zhao et al., 2014a, 2014b), but also imparted them with analyte binding-induced fluorescence quenching properties. These characteristics make them a useful fluorescent chemosensor for direct optosensing of FA in complex biological media, with its limit of detection being 0.025 μM and average recoveries ranging from 98% to 102%.

## 2. Experimental

### 2.1. Materials

Methacrylic acid (MAA, Tianjin Jiangtian Chemicals, 99%, Scheme S1), ethylene glycol dimethacrylate (EGDMA, Alfa Aesar, 98%, Scheme S1), acetonitrile (Tianjin Kangkede Chemicals, Analytical grade (AR)), methanol (Tianjin Jiangtian Chemicals, AR), and azobisisobutyronitrile (AIBN, Chemical Plant of Nankai University, AR) were purified following the literature methods (Ma et al., 2013). Glyceryl monomethacrylate (GMMA, Scheme S1) was synthesized by opening the epoxide ring of glycidyl methacrylate with perchloric acid following the previously reported procedure (van Dijk-Wolthuis et al., 1995). Cumyl dithiobenzoate (CDB, Scheme S1) was obtained by the first synthesis of benzodithioic acid through the reaction of phenylmagnesium bromide and carbon disulfide and its subsequent reaction with $\alpha$-methylstyrene according to the previously reported method (Le et al., 1998). Octadecyl-*p*-vinylbenzyldimethylammonium chloride (OVDAC, Scheme S1) was prepared by reacting *N,N*-dimethyloctadecylamine with 4-vinylbenzyl chloride in acetone under reflux condition following a literature procedure (Zhang et al., 2014). Well-defined PGMMA with a dithioester end group ($M_{n,NMR}$=8180) (i.e., PGMMA macro-CTA, Scheme S1) was synthesized as described in the Supporting Information (Table S1, Fig. S1). An aqueous solution of 3-mercaptopropionic acid-capped CdTe QDs was also prepared as described in the Supporting information. Tetracycline (Tc) hydrochloride (Heowns Biochem Technologies, LLC, Tianjin, 97%) was converted into its neutralized form (Scheme S1) prior to use following a literature method (Schweitz et al., 2000). The standard fetal bovine serum (pH=7.4, Beijing Solarbio Science & Technology Co., Ltd.) and porcine serum (pH=7.5, Tianjin Dingguo Biological Technology Co., Ltd.) were stored at −20 °C prior to use and the thawed serums were directly used in our study. Folic acid (FA, Tianjin Guangfu Fine Chemical Research Institute, 97%, Scheme S1), methotrexate hydrate (MTX, Shanghai Leibo Chemical Technology Co., Ltd, 98%, Scheme S1), trimethoprim (TMP, Shanghai Leibo Chemical Technology Co., Ltd, 99%, Scheme S1), ascrobic acid (VC, Aladdin Industrial Corporation, 99%, Scheme S1), glucose (Heowns Biochem Technologies, LLC, Tianjin, 99%), L-glutamic acid (Beijing Aoboxing Biochem Technologies, LLC, 98%), L-cysteine (Tianjin Guangfu Fine Chemical Research Institute, 99%), bovine serum




**Scheme 1.** Schematic illustration for the one-pot synthesis of QD-labeled hydrophilic FA-MIP nanoparticles for direct and rapid FA optosensing in real, undiluted biological samples.