

**A rapid and high-throughput quantum dots bioassay for monitoring of perfluorooctane sulfonate in environmental water samples**

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**Abstract:** Currently HPLC/MS is the state of the art tool for environmental/drinking water perfluorooctane sulfonate(PFOS) monitoring. PFOS can bind to peroxisomal proliferator-activated receptor-alpha (PPAR $\alpha$ ), which forms heterodimers with retinoid X receptors (RXRs) and binds to PPAR response elements. In this bioassay free PFOS in water samples competes with immobilized PFOS in ELISA plates for a given amount of PPAR $\alpha$ -RXR $\alpha$ . It can be determined indirectly by immobilizing PPAR $\alpha$ -RXR $\alpha$ -PFOS complex to another plate coated with PPAR $\alpha$  antibody and subsequent measuring level of PPAR $\alpha$ -RXR $\alpha$  by using biotin-modified PPAR $\alpha$ -RXR $\alpha$  probes-quantum dots- streptavidin detection system. The rapid and high-throughput bioassay demonstrated a detection limit of 2.5 ng/l with linear range between 2.5 ng/l – 75 ng/l. Detection results of environmental water samples were highly consistent between the bioassay and HPLC/MS.

**Capsule:** We developed a rapid and high-throughput bioassay for monitoring of PFOS in environmental water samples.

**Key words:** High-throughput; Monitoring; Quantum dots Bioassay; Perfluorooctane sulfonate.

## 1. Introduction

Perfluorooctane sulfonate (PFOS) is a well known member of the large family of perfluorinated chemicals (PFCs), which are characterized by a fully fluorinated hydrophobic linear carbon chain attached to various hydrophilic heads. PFOS is also a final degradation product of a group of precursor PFCs (Lau et al., 2007). PFCs have been manufactured for over 50 years. As surfactants and surface protectors, these chemicals have been used as coatings in a wide range of industry and consumer products, including paper, carpets, leather, food containers and fabric. PFCs also act as performance chemicals in products such as fire-fighting foams, floor polishes, and shampoos (Renner, 2001; Olsen et al., 2005).

Owing to the presence of the strong C-F bonds in their chemical structures, PFCs are stable against many chemical reactions and non-biodegradable in the environment and in activated sewage sludge (Brooke, 2004). The total global emission of PFOS is estimated to be 6.5-130 tones with 98% being in water and 2% in air (Rumsby et al., 2009). It has been reported that the level of PFOS in the rivers in China ranged from less than 1 ng/l to greater than 20 ng/l, reflecting the difference in environmental release from different regions (So et al., 2007; Chen, 2009). The samples from the river near Shanghai have the highest PFOS level, which is in concordance with the fact that there are many PFC manufacturers/users in and around Shanghai (Chen, 2009). The levels of PFCs in the lakes/rivers and drink waters are very similar, indicating the ineffectiveness of current water treatment in removing these

chemicals(Rumsby et al., 2009).

PFCs have been shown to accumulate in animals including humans. A mean elimination half-life of 5.4 years has been reported(Olsen et al., 2007). The blood concentration of PFOS varies greatly between different regions and countries. It appeared that among the several countries, China has the highest blood concentration of PFOS(Chen, 2009). Several PFCs including PFOS can bind to and therefore activate the peroxisomal proliferator-activated receptor-alpha (PPAR $\alpha$ ) (of human, rat and mouse), which forms heterodimers with retinoid X receptors (RXRs) and binds to PPAR response elements (PPREs) within the promoter regions of many genes. These genes are involved in a number of biological processes including lipid metabolism and utilization, inflammation, fetal growth, hormone and immune function(Rosen et al., 2009). They may also play roles in cancer development(Lau et al., 2007).

Based on its environmental behavior and human health effect, PFOS has been classified as very persistent, very bioaccumulative and toxic by the EU. Consequently, the use of PFOS has been restricted and will eventually end in EU countries as early as practical(2006a). Similar regulatory measures have also been taken in the United States(2002; 2006b). Nevertheless, the wide and persistent presence of PFOS in the environment presents a continuous threat to human health particularly in China, where the activities in manufacturing and use of PFCs have increased during the last two decade(Chen, 2009). Continues monitoring of PFOS in environmental waters is necessary for evidence based policy making to protect environment and human health.

Most conducted PFC/PFOS monitoring programmes have mainly relied on the

application of high performance liquid chromatography coupled with mass spectrometry (HPLC/MS). Although current state of the art HPLC/MS has detection limits well below 1 ng/l as summarized by (Zhang et al., 2010), the need for accessing to the immobile and expensive equipments has hampered its use in a wide range of conditions. More convenient, cost effective and high throughput alternatives are desirable for large scale environmental water monitoring. The aim of the present study was to develop a bioassay for detecting and monitoring the concentration of PFOS in environmental water, based on the interaction of PFOS with PPAR $\alpha$ -RXR $\alpha$  combined with the use of quantum dots (QD) nanoparticles as a fluorescent marker. The QD were conjugated with streptavidin (SA) that can bind to the PPAR $\alpha$ -RXR $\alpha$  probes labeled with biotin.

Although the sensitivity of the bioassay is lower compared with HPLC/MS, it is convenient, low cost, and allows rapid and high throughput detection. Such detection method is particularly useful for rapid screening for samples with concentration of PFOS higher than 2.5 ng/l.

## **2. Materials and methods**

### *2.1 Reagents and chemicals*

All HPLC purified oligonucleotide probes labeled by biotins were synthesized by Sangon Biotechnology Inc. (Shanghai, China). Quantum dots streptavidin conjugate

(QD-SA) kit was purchased from JiaYuan Quantum Dots Co., Ltd (Wuhan, China). QD-SA was diluted at 1:1000 in diluting buffer and stored at 4°C until use. PFOS was purchased from Fluka (Buchs, Switzerland). Bovine serum albumin (BSA) and dextran coated charcoal were purchased from Sigma-Aldrich Co. (St. Louis, MO, USA). Rabbit polyclonal PPAR $\alpha$  antibody (SC-9000) was purchased from Santa Cruz Biotechnology (Santa Cruz, CA, USA). All reagents for nuclear extraction were obtained from Roche Ltd. (Mannheim, Germany). Ultra-pure water (18 M $\Omega$ , Barnstead International) was used to prepare all the solutions. Black 96-microwell plates were purchased from Nunc Co., Ltd (Denmark).

## *2.2 Preparation of PPAR $\alpha$ -RXR $\alpha$*

The PPAR $\alpha$ -RXR $\alpha$  was extracted from the liver of male Sprague Dawley rat according to the protocol described by Crinelli (Baptista et al., 2006). 20  $\mu$ l aliquots of nuclear extracts were stored at -80°C until use.

## *2.3 Preparation of biotin -modified probes for PPAR $\alpha$ -RXR $\alpha$ binding*

A pair of DNA probes tagged with biotin were designed and synthesized according to the PPAR $\alpha$ -RXR $\alpha$  heterodimer binding corequence (sense: biotin-5'-AAAACTGGGTCAAAGGTCT-3', antisense: biotin-5'-AGACCTTTGACCCAGTTTTT-3'')(Juge-Aubry et al., 1997). The

synthetic single-stranded probes were dissolved in sterile TE buffer (10 mM Tris-HCL, 1 mM EDTA, pH=8.0) and melted in annealing buffer (10 mM Tris-HCL, 1 mM EDTA, 50 mM NaCl, pH 8.0) at 72°C for 3 min, followed by cooling to 60°C for 1.5 min, further to 45°C for 1 min and 10°C for 3 min in a thermal cycler(Wang et al., 2003; Li et al., 2006).

#### *2.4 PFOS-BSA and PPAR $\alpha$ antibody coating in 96 -microwell plates*

PFOS-BSA was prepared according to the methods as described previously(Jones et al., 2003). PFOS and BSA were first mixed at concentrations of 1 mM and 1.2 mM respectively for 2 hr and then 0.025% of dextran coated charcoal was added in order to absorb the free PFOS. After 30 min, the mixture was centrifuged at 10000 *g* for 10 min and the supernatant, which contained PFOS-BSA at concentration of 0.5 mM, was collected. For coating with PFOS-BSA, 96-well black plates were filled with 200  $\mu$ l/well PFOS-BSA diluted to 1  $\mu$ M in coating buffer (NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>, 50 mM, pH=9.6). The plates were stored at 4°C for 12 hr before the supernatants were removed and washed with PBS-Tween 20 (8 mM, pH=7.6, 0.05% Tween 20) for 3 times. The plates were also treated with blocking Buffer (1% BSA) for 2 hr.

For coating with PPAR $\alpha$  antibody, the 96-well plates were filled with 200  $\mu$ l/well antibody at dilution of 1:400 in coating buffer (NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub>, 50 mM, pH=9.6). The rest of the coating procedure was the same as described above.

## *2.5 Quantum dots bioassay for PFOS detection*

PFOS standards were made by dissolving PFOS in the pure water containing 0.05% DMSO at concentrations of 2.5 ng/l, 5 ng/l, 12.5 ng/l, 40 ng/l, 50 ng/l , and 75 ng/l. The solution without PFOS was used as control. 200  $\mu$ l of each of the PFOS standards and 2  $\mu$ l of PPAR $\alpha$ -RXR $\alpha$  extract were added to the 96-well plate coated with PFOS-BSA. The plate was placed on a shaker for 1.5 hr at 37°C to allow full combination of both free PFOS and PFOS-BSA with PPAR $\alpha$ -RXR $\alpha$ . The supernatants that containing the complex of PPAR $\alpha$ -RXR $\alpha$ -PFOS were then transferred to another plate coated with PPAR $\alpha$  antibody. The plate was incubated for 1.5 hr at 37°C to allow the reaction of PPAR $\alpha$ -RXR $\alpha$ -PFOS with the antibody. After discarding the supernatants and washing the plate with PBS-Tween 20 for 3 times, 100  $\mu$ l /well 2 $\times$ nucleoprotein binding buffer and 75  $\mu$ l /well Ultra-pure water were added to the plate. The plate was incubated for 30 min before 25  $\mu$ l of biotin labeled DNA probe (100 mM ) was added to each well. The plate was incubated for 1 hr at 37°C to allow the probe to bind with PPAR $\alpha$ -RXR $\alpha$ -PFOS. The QDs-SA was diluted at 1:1000 in TBS and 200  $\mu$ l/well added. After 2 hr incubation at 4°C, the result was processed by an automatic fluorescence microplate reader at excitation and emission wavelengths of 400 nm and 605 nm, respectively, according to the peak value of the QDs' absorption and emission spectra **(Fig.1)**.

## *2.6 PFOS detection by HPLC/MS*

PFOS standards were separated and quantified using an Agilent 1100 series HPLC/MSD system modified with low dead volume tubing. Separation of compounds was performed using a 150× 2.1mm (5 µm) Zorbax Extend C18 column (Agilent Technologies, U.S.A). A 5 µl aliquot of the sample extract was injected into a guard column (XDB-C8, 2.1mm i.d.×12.5mm, 5 µm; Agilent Technologies, U.S.A) that was sequentially connected to Zorbax Extend C18 column with 1 mM ammonium acetate solution (solvent A) and methanol (solvent B) as mobile phases, starting at 40% of solvent B with flow rate of 0.2 ml/min. At 2 min the solvent B concentration was increased to 60% and held till 5 min when its concentration was increased to 75%, which was held until 11.5 min. The column temperature was kept constant at 40°C.

PFOS standards were identified and quantified using selective ion monitoring (SIM) operating in negative electrospray ionization mode. The ion selected for monitoring in this study was PFOS ( $m/z$  499). Retention times for PFOS were between 9.5 and 10.5 min. The desolvation gas temperature was 350°C at a flow rate of 10 L/min. The collision cell was maintained at 30 psig. Capillary voltage was set at 1500 V with a fragmentor voltage of 70 V. A dwell time of 0.8 s was used to monitor the ions. Correlation between peak areas and analyte concentrations (2.5-75 ng/l) was determined by linear regression with a relative correlation coefficient of 0.9978.

## *2.7 Recovery experiments*

The recovery experiments were conducted by spiking water samples with PFOS standard. The water samples were then analyzed by Bioassay and by HPLC/MS using the same detection parameters as used for analysis of standard samples. The experiments were repeated for 8 times and the recovery rates were calculated by comparing the concentrations derived from spiking samples and the standard concentration.

### *2.8 Collection and pretreatment of environmental water samples*

To minimize the adsorption of PFOS, plastic bottles made of polyethylene were used for sampling. The containers for sample collection were washed with n-hexane, petroleum ether and methanol before use according to GB/T5750.2-2006. Environmental water samples were collected from the Yangtze River (Jiang'an District Wuhan, China), Han River in Wuhan, waterworks in Wuhan and bottled purified water in October 2009. The samples were taken at 20 cm below the water surface in middle of Yangtze River and Han River. One little of water sample was pretreated with filtration through 0.22  $\mu\text{m}$  membrane to remove the solids in the water at the speed of 1 drop per second, and then run through the C18 adsorption column with the help of pump so that PFOS in water can be absorbed on the column. The column was washed with 5 ml of methanol, dried by high-purity nitrogen and dissolved in 1 ml of methanol. Due to its high sensitivity, the normal water samples

for bioassay can be only pretreated with filtration through 0.22  $\mu\text{m}$  membrane to remove the solids without **the enrichment by Solid-Phase Extraction**.

### *2.9 Quantification of PFOS in environmental water samples with quantum dots bioassay and HPLC/MS*

To assess the reliability of the newly developed bioassay, the level of PFOS in water samples was analyzed by the bioassay and compared with that derived with HPLC/MS. Both assays were performed following the procedures as described above.

## **3. Results**

### *3.1 Scheme of the quantum dots bioassay*

Basic principle of this bioassay is shown in **Fig.2**. In this system, the free PFOS in water samples will compete with the immobilized PFOS in 96-well ELISA plate for a given amount of PPAR $\alpha$ -RXR $\alpha$  (Fig 2.1 and 2.2). The level of the free PFOS in the samples can then be determined indirectly by immobilizing the PPAR $\alpha$ -RXR $\alpha$ -PFOS complex to another 96-well plate coated with PPAR $\alpha$  antibody (Fig 2.3) and subsequent measuring the level of PPAR $\alpha$ -RXR $\alpha$  by using the biotin-modified PPAR $\alpha$ -RXR $\alpha$  probes-QD-SA detection system. The amount of PFOS is proportional

to the fluorescence intensity of QDs, therefore PFOS detection can be quantitatively achieved.

### *3.2 Validation of the bioassay for PFOS*

To validate the bioassay system, PFOS standard solutions were measured using both the bioassay and HPLC/MS. In the PFOS bioassay, the intensity of fluorescence from each well was linearly related with the PFOS concentrations ranging from 2.5 ng/l to 75 ng/l ( $y = 19.112x - 23.995$ ,  $R^2 = 0.9642$ ) (Fig.3). The intensity of fluorescence from blank control and standard concentration of 2.5ng/l was 0.35 and 1.31, respectively. The value of S/N was about 3, so the detection limit of this method was 2.5 ng/l with the mean recovery being 107.8% **(Fig.3)**.

In comparison, HPLC/MS demonstrated a limit of detection of 1ng/l and recovery 104.3%. The concentration range from 2.5 ng/l to 75ng/l was within its detection linear range ( $y = 2E-05x - 26.479$ ,  $R^2 = 0.9957$ ) **(Fig.4)**.

**(Table. 1)**.

### *3.3 Quantification of PFOS in environmental water samples with the bioassay and HPLC/MS.*

To further assess the reliability of the bioassay, 3 water samples per place collected were detected for PFOS concentration and the results were compared with

that determined by HPLC/MS as shown in Table 1., suggesting that the novel bioassay is reliable for PFOS quantification. HPLC/MS analysis also confirmed identity of the analyst as PFOS (**Fig.5**).

(**Table.2**).

#### **4. Discussion**

In the present study, a novel bioassay has been successfully developed for detection of PFOS in environmental water samples. In order to achieve reasonably high sensitivity, QD nanoparticles were used to enhance the signals for detection in the bioassay system. Over traditional fluorophores, QD nanoparticles have several advantages that include a long fluorescent lifetime, high fluorescent intensity(Ghasemi et al., 2009), broad excitation spectrum, narrow emission spectrum, and large separation between the excitation and emission wavelength(Baptista et al., 2006; Kjallman, 2008).

This study was initially designed for detection of PFOS, the method however can also be applied for detection of several other PFCs that can bind to the PPAR $\alpha$ . Although the sensitivity of the bioassay is lower than HPLC/MS, the QDs bioassay is rapid, convenient and high-throughput. It is particularly useful as a tool for rapid screening for environmental water samples that contain high level concentration of PFCs. The actual identities and concentration of the detected PFCs can be further confirmed using HPLC/MS. The QDs bioassay can also be used to monitor large

number of residential drinking water samples for PFOS content and evaluate the effectiveness of PFOS-rich sewage treatment. If the concentration of PFOS in sewage samples is higher than 75ng/L, we can dilute them to 1/10,1/100,1:1000 or more before testing, and test them at one time for high-throughput of the bioassay. At last one diluted sample must be in the detection range(2.5~75ng/L), then we can calculate the concentration according to the sample's dilution. Such detection method can therefore serve as a complementary or an alternative tool to HPLC/MS for PFC detection and monitoring in environmental waters.

The increase in manufacturing and application of PFOS and related chemicals in China during the last two decades has inevitably increased the release of these chemicals into environmental waters. In some areas, especially in regions with intensive industrial activities, the level usually exceeds above average. The level of PFOS has been detected even greater than 100 ng/l in Pear River near the city of Dongguan Guangdong province of China(So et al., 2007). The industries in this city were heavily involved in the production of plastics, electrical equipments and telecommunication equipments developed during 1980 and 1997(Nolan, 2001). Many of these industries could potentially involve the use of PFOS and other PFCs during their manufacturing processes. The level of PFOS in drinking water in Shanghai, the most important special economic zone of china, was also found to be higher than 10 ng/l(So et al., 2007).

Current water purification practices, including slow sand filtration and chlorination, were ineffective in removing PFOS(Loos et al., 2007). Other methods

have also been tried to increase the effectiveness of PFOS removing. Tang et al. investigated the use of reverse osmosis (RO) membranes and nanofiltration (NF) membranes to remove PFOS from wastewater. A greater than 99% and 90%–99% removal was achieved with RO and NF membranes, respectively (Tang et al., 2006; Tang et al., 2007). With the installation of granular activated carbon (GAC) system into the wastewater treatment system of the PFOS production facility in Minnesota, PFOS was successfully removed from the effluent discharged into the Mississippi River. These new technologies, however, have not currently been well implemented.

Combined with the ineffective wastewater treatment, the increase in the manufacturing and use of PFOS and related chemicals pose a continuous threat to the environment and human health. Taken together, rigorous monitoring of PFOS in environmental and drinking waters with rapid, cost-effective and robust tool can help to evaluate the level of environment contamination and the effectiveness of water treatment practice in PFOS and related chemical industries, benefiting both environment and human health.

## **5. Conclusion**

We have developed a QDs bioassay for convenient, cost effective and robust detection of PFOS in water samples. This bioassay has detection limit of 2.5ng/l with linearity range of 2.5ng/l-75ng/l, providing an alternative to HPLC/MS for detection and monitoring of PFOS in environmental water and drinking water, and also for

evaluating the effectiveness of water treatment for removing PFOS and related chemicals.

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## References

2002. 3M Phase-out Plan for POSF-Based Products. United States Environmental Protection Agency, Washington, DC.
- 2006a. Directive 2006/122/ECOF of the European Parliament and of the Council of 12 December 2006, Official Journal of the European Union. European Union, pp. 32-34.
- 2006b. Perfluorooctane sulfonate and its salts and certain other compounds regulations. Government of Canada, pp. 4265-4284.
- Baptista, P.V., Koziol-Montewka, M., Paluch-Oles, J., Doria, G., Franco, R., 2006. Gold-nanoparticle-probe-based assay for rapid and direct detection of *Mycobacterium tuberculosis* DNA in clinical samples. *Clinical Chemistry* 52, 1433-1434.
- Brooke, D.F.A.N., 2004. Environmental risk evaluation report: Perfluorooctanesulfonate . Environment Agency, UK.
- Chen, C.L.Y.L., 2009. A review of spatial and temporal assessment of PFOS and PFOA contamination in China. *Chemistry and Ecology* 25, 163-177.
- Ghasemi, Y., Peymani, P., Afifi, S., 2009. Quantum dot: magic nanoparticle for imaging, detection and targeting. *Acta Biomed* 80, 156-165.
- Jones, P.D., Hu, W., De Coen, W., Newsted, J.L., Giesy, J.P., 2003. Binding of perfluorinated fatty acids to serum proteins. *Environmental Toxicology And Chemistry* 22, 2639-2649.
- Juge-Aubry, C., Pernin, A., Favez, T., Burger, A.G., Wahli, W., Meier, C.A., Desvergne, B., 1997. DNA binding properties of peroxisome proliferator-activated receptor subtypes on various natural peroxisome proliferator response elements. Importance of the 5'-flanking region. *Journal Of Biological Chemistry* 272, 25252-25259.
- Kjallman, T.H.P., 2008. Quenching of an indocarbocyanine dye and functionalized CdSe/ZnS quantum dots by gold surfaces. *Current Applied Physics* 8, 308-311.
- Lau, C., Anitole, K., Hodes, C., Lai, D., Pfahles-Hutchens, A., Seed, J., 2007. Perfluoroalkyl acids: a review of monitoring and toxicological findings. *Toxicological Sciences* 99, 366-394.
- Li, B.S., Zhao, L.F., Zhang, C., Hei, X.H., Li, F., Li, X.B., Shen, J., Li, Y.Y., Huang, Q., Xu, S.Q., 2006. Ultra-sensitive colorimetric method to quantitate hundreds of polynucleotide molecules by gold nanoparticles with silver enhancement. *Analytical Sciences* 22, 1367-1370.
- Loos, R., Wollgast, J., Huber, T., Hanke, G., 2007. Polar herbicides, pharmaceutical products, perfluorooctanesulfonate (PFOS), perfluorooctanoate (PFOA), and nonylphenol and its carboxylates and ethoxylates in surface and tap waters around Lake Maggiore in Northern Italy. *Analytical And Bioanalytical Chemistry* 387, 1469-1478.
- Nolan, P.A.G.Y., 2001. Big business with Chinese characteristics: two paths to growth of the firm in China under reform. *Cambridge Journal of Economics* 25, 443-465.
- Olsen, G.W., Burris, J.M., Ehresman, D.J., Froehlich, J.W., Seacat, A.M., Butenhoff, J.L., Zobel, L.R., 2007. Half-life of serum elimination of perfluorooctanesulfonate, perfluorohexanesulfonate, and perfluorooctanoate in retired fluorochemical production workers. *Environ Health Perspect* 115, 1298-1305.
- Olsen, G.W., Huang, H.Y., Helzlsouer, K.J., Hansen, K.J., Butenhoff, J.L., Mandel, J.H., 2005. Historical comparison of perfluorooctanesulfonate, perfluorooctanoate, and other fluorochemicals in human blood. *Environ Health Perspect* 113, 539-545.

- Renner, R., 2001. Growing concern over perfluorinated chemicals. *Environmental Science & Technology* 35, 154A-160A.
- Rosen, M.B., Schmid, J.E., Das, K.P., Wood, C.R., Zehr, R.D., Lau, C., 2009. Gene expression profiling in the liver and lung of perfluorooctane sulfonate-exposed mouse fetuses: comparison to changes induced by exposure to perfluorooctanoic acid. *Reproductive Toxicology* 27, 278-288.
- Rumsby, P.C., McLaughlin, C.L., Hall, T., 2009. Perfluorooctane sulphonate and perfluorooctanoic acid in drinking and environmental waters. *Philos Transact A Math Phys Eng Sci* 367, 4119-4136.
- So, M.K., Miyake, Y., Yeung, W.Y., Ho, Y.M., Taniyasu, S., Rostkowski, P., Yamashita, N., Zhou, B.S., Shi, X.J., Wang, J.X., Giesy, J.P., Yu, H., Lam, P.K., 2007. Perfluorinated compounds in the Pearl River and Yangtze River of China. *Chemosphere* 68, 2085-2095.
- Tang, C.Y., Fu, Q.S., Criddle, C.S., Leckie, J.O., 2007. Effect of flux (transmembrane pressure) and membrane properties on fouling and rejection of reverse osmosis and nanofiltration membranes treating perfluorooctane sulfonate containing wastewater. *Environmental Science & Technology* 41, 2008-2014.
- Tang, C.Y., Fu, Q.S., Robertson, A.P., Criddle, C.S., Leckie, J.O., 2006. Use of reverse osmosis membranes to remove perfluorooctane sulfonate (PFOS) from semiconductor wastewater. *Environmental Science & Technology* 40, 7343-7349.
- Wang, L.H., Yang, X.Y., Zhang, X., Mihalic, K., Xiao, W., Farrar, W.L., 2003. The cis decoy against the estrogen response element suppresses breast cancer cells via target disrupting c-fos not mitogen-activated protein kinase activity. *Cancer Research* 63, 2046-2051.
- Zhang, X., Niu, H., Pan, Y., Shi, Y., Cai, Y., 2010. Chitosan-coated octadecyl-functionalized magnetite nanoparticles: preparation and application in extraction of trace pollutants from environmental water samples. *Analytical Chemistry* 82, 2363-2371.

## Figure legends:

**Figure 1.** Absorption and emission spectra of QDs-SA

a Absorption spectra of QDs-SA.                      b. Emission spectra of QDs-SA.

**Figure2.** Schematic illustration of the bioassay using QD-streptavidin-biotin-modified oligonucleotide probes.

(1). Plate was coated with PFOS-BSA; (2). PFOS standard/sample and PPAR $\alpha$ -RXR $\alpha$  were added; (3). Supernatants were transferred to another plate coated with PPAR $\alpha$  antibody; (4). Biotin modified probes were added; (5) QDs-SA was added.

**Figure 3.** Quantification of PFOS in standard solutions with the bioassay using QD-streptavidin-biotin-modified oligonucleotide probes. The curve shows the linearity between fluorescence intensities against corresponding concentrations of PFOS. Each value represents the mean  $\pm$  SD of triplicate independent determinations. 0.05%DMSO was used as a control.

**Figure 4.** Quantification of PFOS in standard solutions with HPLC/MS. The curve shows the linearity between absorption peaks against corresponding concentrations of PFOS. Each value represents the mean  $\pm$  SD of triplicate independent determinations. 0.05%DMSO was used as a control.

**Figure 5.** Representative HPLC/MS spectra of PFOS in environmental water samples. Environmental water samples were prepared and analyzed for PFOS by HPLC/MS as described in the materials and methods section.

a. PFOS of chromatogram;    b. PFOS of mass spectrogram

**Table.1** Analytical parameters of the two methods for PFOS quantification

<sup>1</sup>The Detection limits were calculated by S/N=3

**Table.2** Quantification of PFOS in environmental water samples with the bioassay and HPLC/MS



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