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# Streptavidin sensor and its sensing mechanism based on water-soluble fluorescence conjugated polymer

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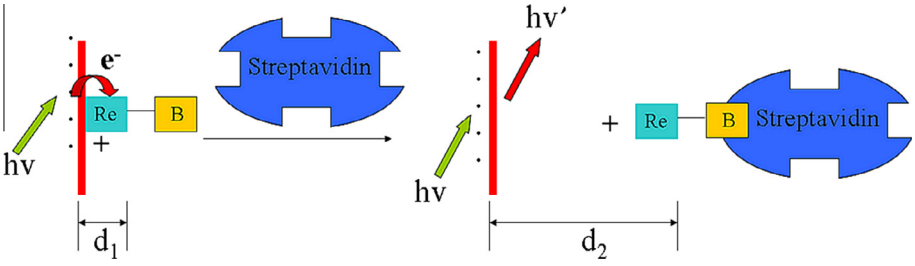
## HIGHLIGHTS

- The sensing mechanism was stable whether in non-buffer system or buffer system.
- Re-Biotin probe is superior to $BPP^+$ probe.
- Strptavidin or avidin can be classified from other proteins by Re-Biotin.
- Strptavidin or avidin can be quantitatively determinated by Re-Biotin.

## GRAPHICAL ABSTRACT

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## ABSTRACT

Fluorescence quenching effect of water-soluble anionic conjugated polymer (CP) (poly[5-methoxy-2-(3-sulfopoxy)-1,4-phenylenevinylene] (MPS-PPV)) by [Re(N-N)$(CO)_3$(py-$CH_2$-NH-biotin)]$(PF_6)$ [N-N=2,9-dimethyl-4,7-diphenyl-1,10-phenanthroline; py-$CH_2$-NH-biotin=N-[(4-pyridyl) methyl] biotinamide] (Re-Biotin) and fluorescence recovery in the presence of streptavidin (or avidin) were investigated using Re-Biotin as quencher tether ligand (QTL) probe. Meanwhile, the mechanisms of fluorescence quenching and recovery were discussed to provide new thoughts to design biosensor based on water-soluble CPs. The results indicate that the sensing mechanisms of streptavidin sensor or avidin sensor, using Re-Biotin as QTL probe, are the same and stable, whether in non-buffer system (aqueous solution) or different buffer systems [0.01 $mol \cdot L^{-1}$ phosphate buffered solution (pH = 7.4), 0.1 $mol \cdot L^{-1}$ ammonium carbonate buffered solution (pH = 8.9)]. There exists specific interactions between streptavidin (or avidin) and biotin of Re-Biotin. Fluorescence quenching and recovery processes of MPS-PPV are reversible. Mechanisms of Re-Biotin quenching MPS-PPV fluorescence can be interpreted as strong electrostatic interactions and charge transferences between Re-Biotin and MPS-PPV. Fluorescence recovery mechanisms of Re-Biotin—MPS-PPV system can be interpreted as specific interactions between streptavidin (or avidin) and biotin of Re-Biotin making Re-Biotin far away from MPS-PPV. Avidin or strptavidin as re-Biotin probe can not only be quantitatively determinated, but also be identified.



## Introduction

In 1999, a new bioprobe technique (quencher tether ligand, QTL) based on water-soluble fluorescence conjugated polymers (CPs) was establish in non-buffer system by Whitten et al. [1] from the US Department of Energy (DOE), Los Alamos National Laboratory, selecting biotin–avidin as ligand receptor theoretical model, and a high sensitive biosensor detecting avidin was synthesized. Since 2000, biosensor develops rapidly using water-soluble fluorescence CPs as sensing materials. At present, it has become the hotspot of biosensor area. Many scientists had summarized the synthesis, properties and applications of some water-soluble fluorescence CPs in the field of chemical and biological sensor [2–9]. Wang et al. [8,9] had summarized the research progresses of

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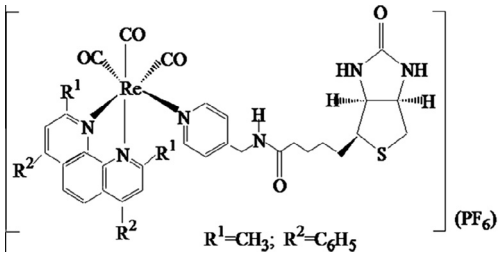
water-soluble fluorescence CPs in DNA, protein detection and sensing, cell and cell fluorescence imaging, construction of biological devices and so on. Furthermore, they forecasted that there must be significant application in high sensitive diagnosis of early stage of serious disease, drug design and individualized therapy.

At present, water-soluble fluorescence CPs have been applied in DNA detection [3,6,8,10–26], DNA and enzymes [27], RNA–proteins [28], proteins [29–33], antibodies of dinitrophenol derivative [34], enzymes and enzyme activities [35–43], cell [44–47] and cell fluorescence imaging [48–54] and so on. Although water-soluble fluorescence CPs have been used as sensitive materials to detect the biomacromolecular sensors above, their sensing mechanisms have not been very clear. Dwight et al. [29] studied the action mechanism among water-soluble anionic CP (poly[5-methoxy-2-(3-sulfopoxy)-1,4-phenylenevinylene] (MPS-PPV)), biotinylated quenching agent Q-B [N-(biotinoyl)-N′-(acetyl 4,4′-pyridylpyridinium iodide)] ethylenediamine ($BPP^+$)) and avidin in non-buffer system (aqueous solution) and buffer system (ammonium carbonate buffered solution of pH = 8.9). Two sensing models were put forward. In non-buffer system (aqueous solution), there is strong fluorescence quenching of MPS-PPV by Q-B in the absence of avidin, while it can make Q-B far away from MPS-PPV in the presence of avidin, so that the fluorescence of MPS-PPV can recover, which is similar with the QTL model designed by Whitten et al. [1]. While in the ammonium carbonate buffered solution of pH = 8.9, there is weak fluorescence quenching of MPS-PPV by Q-B in the absence of avidin, and the fluorescence quenching will be weaker in the presence of avidin, which is different from the QTL model designed by Whitten et al. Why the action mechanisms among water-soluble anion CP MPS-PPV, biotinylated quenching agent Q-B and avidin are different in non-buffer system (aqueous solution) and buffer system (ammonium carbonate buffered solution of pH = 8.9)? Whether other buffer systems are the same as ammonium carbonate buffer system? What are the action mechanisms among different biotinylated quenching agent Q-B, MPS-PPV and avidin?

Streptavidin (SA) is a kind of protein which has the similar biological property with avidin (A). In practical application, the detection sensitivity of SA is obviously superior to that of A [55]. SA has the same properties and application utility with A. One molecular SA or A can combine with 4 moleculars biotin. Therefore, biotin streptavidin system is constantly used to prepare biosensors just as biotin avidin system (BAS).

The application of MPS-PPV in polylysine [56], NO sensor [57] has been reported, as well as the influence of MPS-PPV fluorescence by various surfactants and metal ions [58]. Then the sensors used for rapidly detecting noble metal $Pd^{2+}$, $Ru^{3+}$, $Pt^{2+}$ based on MPS-PPV were prepared [59].

In this article, streptavidin sensor was prepared based on water-soluble CP as sensitive material. In the experiments, biotinylated Re (I) complex—[Re(N-N)$(CO)_3$ (py-$CH_2$-NH-biotin)]($PF_6$) [N-N=2, 9-dimethyl-4,7-diphenyl-1,10-phenanthroline; py-$CH_2$-NH-biotin=N-[(4-pyridyl) methyl] biotinamide] (Re-Biotin, Scheme 1) was used as QTL probe. The experiments were completed according to the fluorescence quenching effect of MPS-PPV by Re-Biotin and fluorescence recovery characteristic in the presence of streptavidin (or avidin). And the mechanism of fluorescence quenching and recovery was discussed. The results show that the sensing mechanisms of streptavidin sensor and that of avidin sensor are the same and stable, whether in non-buffer system (aqueous solution) or different buffer systems [0.01 mol·$L^{-1}$ phosphate buffered solution (pH = 7.4), 0.1 mol·$L^{-1}$ ammonium carbonate buffered solution (pH = 8.9)].




**Scheme 1.** Chemical structure of Re-Biotin.

## Experimental

### *Apparatus and reagents*

The pH was recorded with a PHS-3C precision pH meter. The fluorescence emission spectra were measured on a LS 55 luminescence spectrometer (Perkin Elmer Co.). MPS-PPV was prepared according to Ref. [60]. Re-Biotin [61] was offered by Dr. Jinrong Luo from City University of Hong Kong. Streptavidin, avidin, bovine serum albumin (BSA) and protamine sulfate (PS) were purchased from Sigama Co. and other reagents were domestic analytical reagents. Buffered solutions were 0.01 mol·$L^{-1}$ phosphate buffered solution (PBS, pH = 7.4) and 0.1 mol·$L^{-1}$ ammonium carbonate buffered solution (pH = 8.9). The Milli-Q water was used as pure water.

### *Fluorescence spectra measurement*

The fluorescence intensity $I_0$ was measured on a LS 55 luminascence spectrometer (Perkin Elmer Co.). MPS-PPV solutions were added into fluorescence cuvette, and the fluorescence spectra were recorded with excitation at 387 nm and emission at 496 nm. Under the same condition, the fluorescence intensities $I$ were recorded while adding various concentrations of Re-Biotin and the mixture solutions of Re-Biotin and streptavidin (or avidin or other proteins) into MPS-PPV solutions.

## Results and discussion

### *Fluorescence quenching effect of MPS-PPV by Re-Biotin and fluorescence potentiation of Re-Biotin—MPS-PPV System by streptavidin in phosphate buffered solution (pH = 7.4)*

Fig. 1 is the fluorescence spectrum of $3.0 \times 10^{-5}$ mol·$L^{-1}$ MPS-PPV in the absence and presence of Re-Biotin in 0.01 mol·$L^{-1}$ phosphate buffered solution. With the addition of Re-Biotin and enlargement of its concentration, the fluorescence intensities of the system gradually reduce and the peaks blue shift appear (from 496 nm to 465 nm). It was found that fluorescence of MPS-PPV could be rapidly quenched while trace amounts of Re-Biotin tetrahydrofuran solutions were added to MPS-PPV solution. It indicates that there exist strong electrostatic interactions between Re-Biotin carrying a unit positive charge and anionic polymer MPS-PPV. At the same time, electrons of anionic polymer MPS-PPV feedback to d orbital of Re(I) of Re-Biotin (that is charge transferance). The peaks blue shift may result from scattering peaks shaping from MPS-PPV agglomerating superpose on fluorescence peaks because of electrostatic interactions between Re-Biotin and MPS-PPV. The illustration in Fig. 1 is Stern–Volmer chart of Re-Biotin quenching MPS-PPV. The mechanisms of Re-Biotin quenching MPS-PPV can be interpreted as the strong electrostatic interactions and charge transferance between Re-Biotin and MPS-PPV [1,62,63]. Re-Biotin quenches MPS-PPV fluorescence according to Stern–Volmer equation: