

*Electronic Supplementary Information*

## **Nanoparticle-enhanced fluorescence emission for non-separation assay of carbohydrates using boronic acid-Alizarin complex**

Qianjin Li, Tripta Kamra and Lei Ye \*

Division of Pure and Applied Biochemistry, Lund University, Box 124, 221 00 Lund, Sweden  
Tel: +46 2229560; Fax: +46 2224611; Email: lei.ye@tbiokem.lth.se

### **1. Materials and equipments**

D-fructose, D-glucose, Alizarin red S (ARS), Alizarin, 3-nitrophenylboronic acid (NPBA), 4-vinylpyridine (Vpy), silica nanoparticles (diameter 10 nm), (3-aminopropyl)triethoxysilane (APTES), ethylene glycol dimethacrylate (EGDMA, 98%) and *N,N'*-methylenebisacrylamide (MBAA) were purchased from Sigma-Aldrich (Gillingham, UK). Trimethylolpropane trimethacrylate (TRIM) was purchased from Sigma-Aldrich (Steinheim, Germany). Methacrylic acid (MAA) and azobisisobutyronitrile (AIBN) were purchased from ACROS (Geel, Belgium). Acetonitrile (ACN) and methanol (MeOH) of HPLC grade were from Honeywell (Seelze, Germany). Ethanol (EtOH, 99.7%) was obtained from Solveco (Rosersberg, Sweden). AIBN was re-crystallized from methanol before use. Other solvents and inorganic salts were of analytical reagent grade and were used directly without further purification.

Attenuated total reflection (ATR) infrared spectra were recorded using a Perkin-Elmer FTIR instrument (Perkin-Elmer Instruments). UV absorption spectra were recorded with a Beckman Coulter DU 800 UV/Vis spectrophotometer. Fluorescence emission was measured using a QuantaMaster C-60/2000 spectrofluorometer (Photon Technology International, Lawrenceville, NJ). Scanning electron microscopy (SEM) imaging was carried out on a JEOL JSM-6700F Field Emission Scanning Electron Microscope (Tokyo, Japan). Particle size distribution was measured on a Zetasizer Nano ZS instrument equipped with a software package DTS Ver. 4.10 (Malvern Instruments Ltd., Worcestershire, UK).

### **2. Synthesis of nanoparticles**

Organic particles polyEGDMA, poly(Vpy-co-EGDMA) and polyMBAA were synthesized by precipitation polymerization.<sup>1</sup> Briefly, a pre-polymerization solution containing monomer, crosslinker and initiator in organic solvent was purged with N<sub>2</sub>

gas for 10 min and sealed. The pre-polymerization solution was transferred into a Stovall HO-10 Hybridization Oven (Greensboro, NC, USA) and heated to 60 °C for 24 h while being rotated at a speed of 20 rpm. After polymerization, the particles were collected by centrifugation, washed with methanol for three times and dried in a vacuum chamber at room temperature.

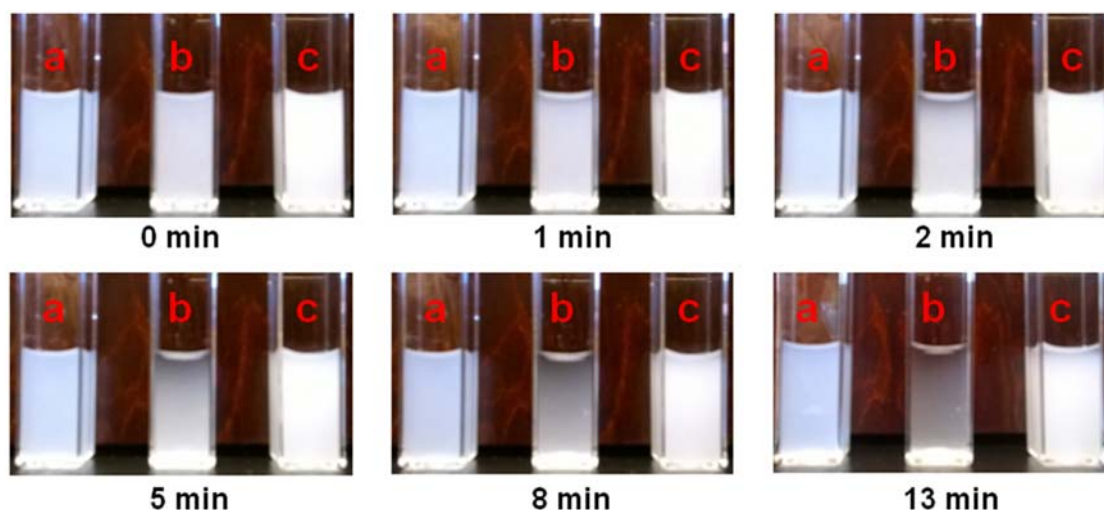
Amino-functionalized silica nanoparticles (Si-NH<sub>2</sub>) were prepared according to a literature method with some minor modification.<sup>2</sup> Briefly, silica nanoparticles (10 nm, 5.4 g) were added into 300 mL toluene and stirred for 30 min. After addition of 18 mL APTES, the mixture was stirred for 2 h at room temperature before it was heated to reflux for 4 h. The obtained Si-NH<sub>2</sub> nanoparticles were collected by centrifugation, washed with MeOH for three times and dried in a vacuum chamber at room temperature.

Carboxyl-functionalized nanoparticles, poly(MAA-co-TRIM), were prepared according to a literature method.<sup>1</sup>

### 3. Particle size measurement

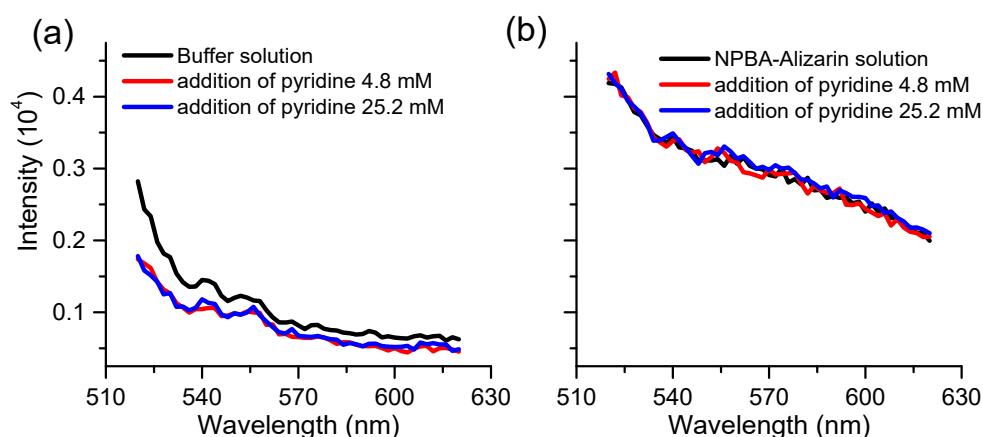
The sizes of the nanoparticles were measured by dynamic light scattering (DLS) using a Zetasizer Nano ZS instrument. The nanoparticles (2 mg) were dispersed in MeOH or water (2 mL) and diluted to a concentration of 0.2 mg mL<sup>-1</sup> prior to the measurement. The DLS measurement was carried out at 20 °C. Data are reported as hydrodynamic diameter by intensity, and are average values from three independent measurements. The average sizes of the nanoparticles Si-NH<sub>2</sub> and poly(MAA-co-TRIM) were found to be about 173 nm and 193 nm, respectively, in agreement with the values reported in the literature.<sup>1,2</sup>

### 4. Stability of particle suspensions



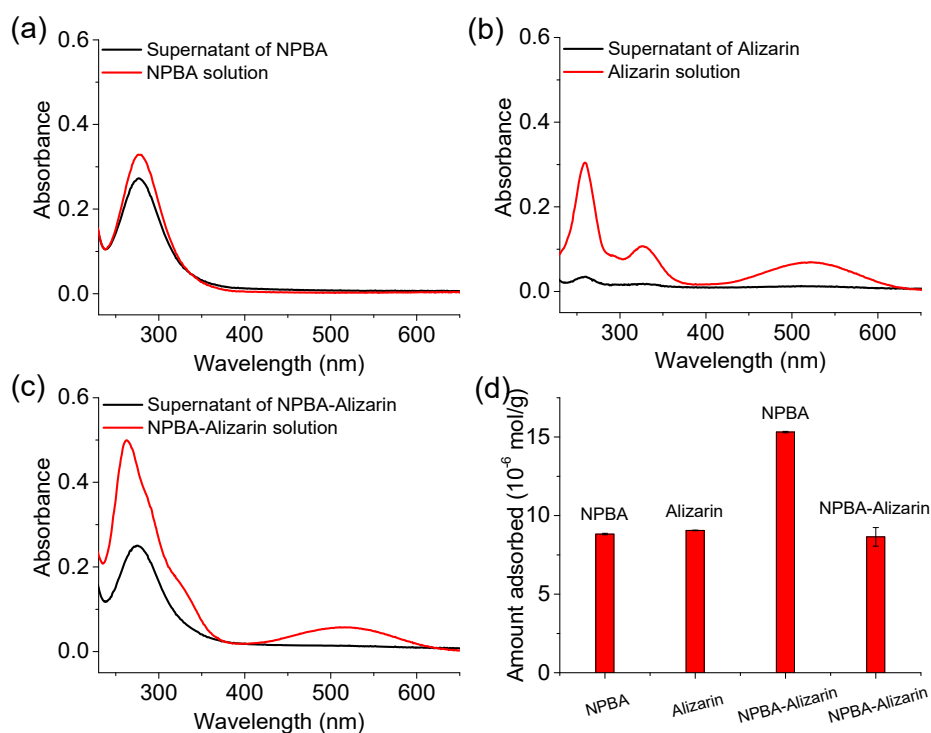
**Figure S1.** Stability of particle suspensions containing poly(Vpy-co-EGDMA) (a), polyEGDMA (b) and polyMBAA (c). Particle concentration: 1.0 mg mL<sup>-1</sup> in sodium phosphate buffer (50 mM, pH 7.4).

## 5. Effect of pyridine on fluorescence emission



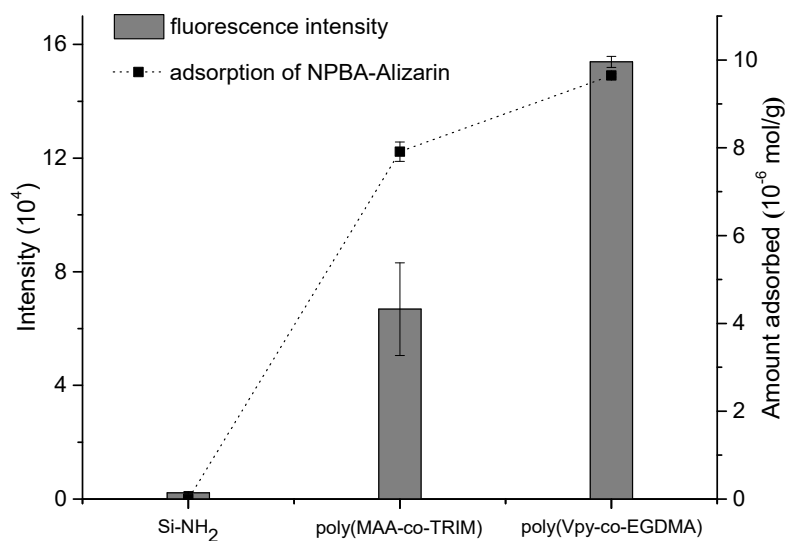
**Figure S2.** Fluorescence spectra of phosphate buffer (50 mM, pH 8.0) (a) and NPBA-Alizarin solution (b) measured in the absence and presence of pyridine at different concentrations. The concentrations of Alizarin, ARS and NPBA are 10  $\mu$ M, 10  $\mu$ M and 50  $\mu$ M, respectively. The excitation wavelength was 467 nm.

## 6. Molecular adsorption by poly(Vpy-co-EGDMA) particles



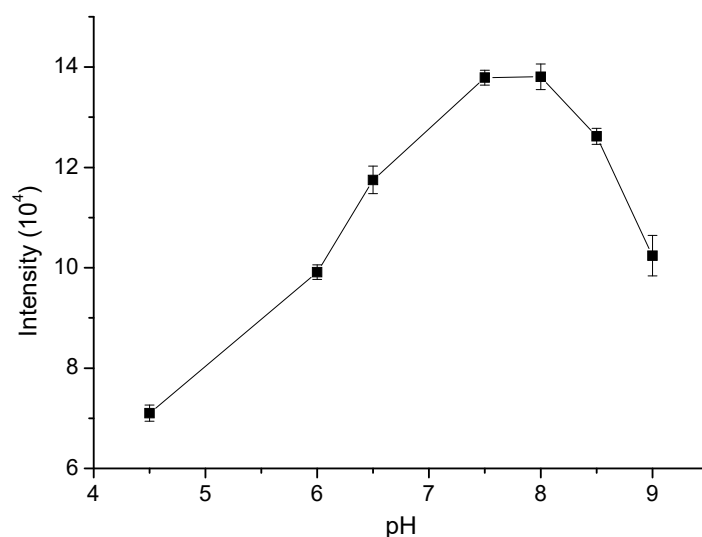
**Figure S3.** UV spectra of solutions NPBA (a), Alizarin (b) and NPBA-Alizarin (c), and the corresponding supernatants after the solutions have been treated with poly(Vpy-co-EGDMA). (d) The amount of molecules (labelled on top of the bars) adsorbed on particle poly(Vpy-co-EGDMA) in different solutions (indicated under the x-axis). Poly(Vpy-co-EGDMA) concentration was 1.0 mg mL<sup>-1</sup> in sodium phosphate buffer (50 mM, pH 7.4). The initial concentrations of NPBA, Alizarin and ARS were 50  $\mu$ M, 10  $\mu$ M and 10  $\mu$ M, respectively. The UV wavelengths used to quantify NPBA, Alizarin, ARS and NPBA-Alizarin were 278, 516, 516 and 516 nm.

## 7. Effect of molecular adsorption on nanoparticles



**Figure S4.** Fluorescence intensity of NPBA-Alizarin solution in the presence of particles Si-NH<sub>2</sub>, poly(MAA-co-TRIM) and poly(Vpy-co-EGDMA), and adsorption of NPBA-Alizarin on the different nanoparticles. NPBA-Alizarin solution is prepared by mixing NPBA (50  $\mu$ M) and Alizarin (10  $\mu$ M) in sodium phosphate buffer (50 mM, pH 7.4). Nanoparticle concentration was 1 mg mL<sup>-1</sup>. Fluorescence intensity was measured at 558 nm using an excitation wavelength at 467 nm.

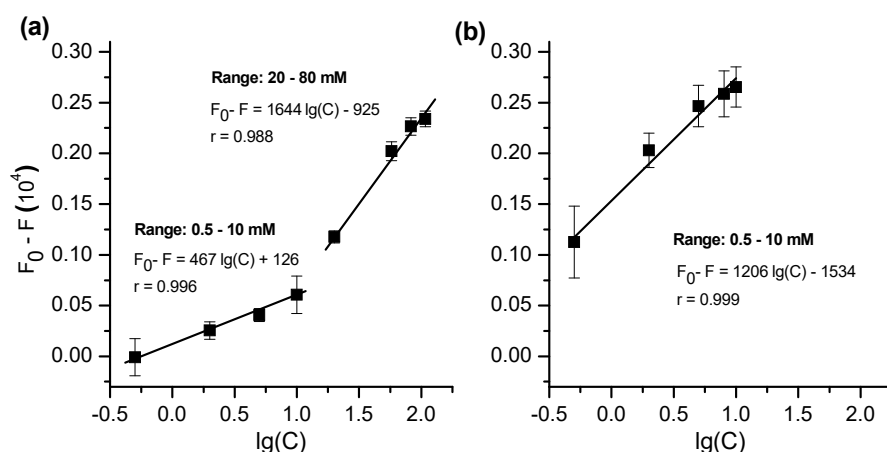
## 8. Effect of pH on fluorescence emission



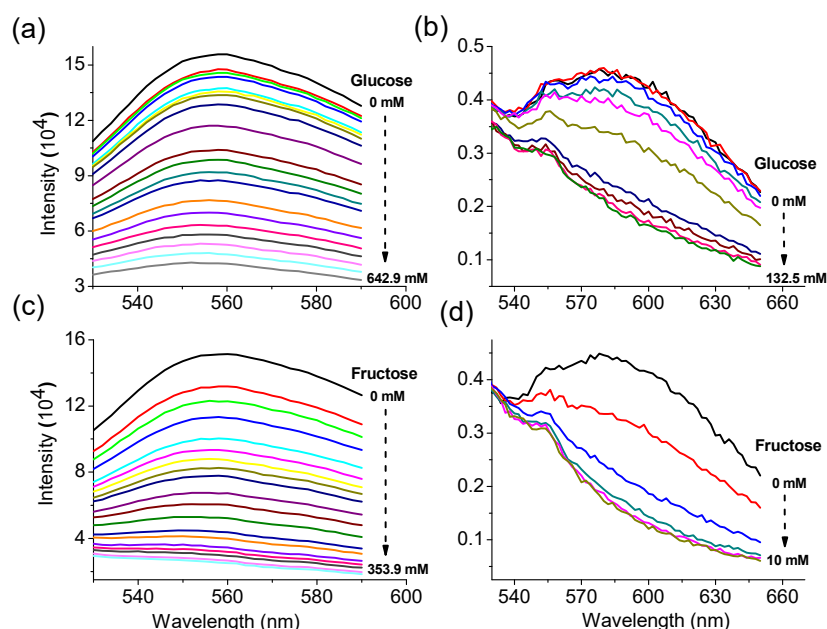
**Figure S5.** Effect of pH on the fluorescence intensity of poly(Vpy-co-EGDMA)-NPBA-Alizarin system. The concentration of Alizarin and NPBA were 10  $\mu$ M and 50  $\mu$ M, respectively. Poly(Vpy-co-EGDMA) concentration was 1 mg mL<sup>-1</sup>. Fluorescence intensity was measured at 558 nm using an excitation wavelength at 467 nm.

## 9. Non-separation carbohydrate assay

A solution of NPBA (50  $\mu\text{M}$ ) and Alizarin (10  $\mu\text{M}$ ) was prepared in phosphate buffer (50 mM, pH 7.4). For the system that involved nanoparticles, the nanoparticles were added into the NPBA-Alizarin solution to give a final particle concentration of 1  $\text{mg mL}^{-1}$ . The fluorescence intensity ( $F_0$ ) of the mixture was measured. After addition of a concentrate sugar solution (2 M), the mixture was shaken for 10 min before the new fluorescence intensity ( $F$ ) was measured.



**Figure S6.** Reduction of fluorescence intensity on NPBA-Alizarin solution caused by glucose (a) and fructose (b).  $F_0$  and  $F$  are the fluorescence intensities of the NPBA-Alizarin solution measured in the absence and presence of the carbohydrates, respectively. The fluorescence intensity was measured at 558 nm using an excitation wavelength at 467 nm.  $C$ , carbohydrate concentration.



**Figure S7.** Fluorescent spectra of NPBA-Alizarin solution in the presence (a and c) and absence (b and d) of poly(Vpy-co-EGDMA) nanoparticles measured after addition of different amount of glucose (a and b) and fructose (c and d). The concentrations of Alizarin and NPBA were 10  $\mu\text{M}$  and 50  $\mu\text{M}$ , respectively. The excitation wavelength used was 467 nm.

## References

1. K. Yoshimatsu, K. Reimhult, A. Krozer, K. Mosbach, K. Sode and L. Ye, *Anal. Chim. Acta*, 2007, **584**, 112.
2. G. S. Caravajal, D. E. Leyden, G. R. Quinting and G. E. Maciel, *Anal. Chem.*, 1988, **60**, 1776.