

# Expanding the dynamic measurement range for polymeric nanoparticle pH sensors

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## Materials

N,N-methylenebis(acrylamide), acrylamide, N,N,N',N'-tetraethylmethylenediamine (TEMED), ammonium persulfate (APS), polyoxyethylene(4)lauryl ether (Brij30), Triton X-100, 1-hexanol, cyclohexane, Oregon green 488 isothiocyanate (Oregon Green ITC), rhodamine B isothiocyanate (RhB ITC), fluorescein-5-isothiocyanate (FITC), molecular sieves 4Å, and dimethyl sulfoxide were purchased from Sigma-Aldrich. 2',7'-bis-(2-carboxyethyl)-5-(and - 6)-carboxyfluorescein (BCECF) succinimidyl ester and Alexa 633 succinimidyl ester was obtained from Invitrogen. N-(3-aminopropyl)methacrylamide hydrochloride (AAC) was obtained from Polyscience. Dimethyl sulfoxide was dried over molecular sieve 4A. All other reagents were obtained from Sigma-Aldrich without further purification.

## Analysis

### UV Spectroscopy

UV spectra were recorded using a Shimadzu UV-Visible Spectrophotometer (phasmaspec UV-1700). The analysis was performed using Shimadzu UV Probe software.

### Zeta Potential Measurements

Zeta Potential was measured in MilliQ water with a ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments Corporation) at room temperature. Each data point was an average of 10 runs.

### Dynamic Light Scattering Measurements

pH nanosensors (0.5 mg/mL) in milliQ water (1.5 mL) or buffer was subjected to ultrasonic treatment and filtered through a 0.45µm micron syringe filter before measurement. The hydrodynamic radius of the nanoparticles were measured with a

ZetaPALS Zeta Potential Analyzer (Brookhaven Instruments Corporation) at 25°C with a fixed scattering angle of 90°.

### **Fluorescence Spectra**

The fluorescence spectra were measured on FL 920 spectrometer (Edinburgh Instruments). The samples were excited at 488nm (fluorescein, BCECF and Oregon Green) and 545nm (Rhodamine B) in a quartz cuvette at room temperature. The dwell time was 0.2s and the sample was scanned twice for each measurement.

### **Experimental procedures**

#### **Preparation of primary amino NPs (NPs-NH<sub>2</sub>)**

2.270g of acrylamide, 0.570g of methylbisacrylamide, and 0.057g of (3-propylamine) methylacrylamine hydrochloride were dissolved in 6.15mL of milliQ water. 8.8mL of this monomer solution was added drop wise to 308mL of oil phase (taken from a stock solution containing 62.5g TX-100, 153.27g 1-hexanol in 1000mL cyclohexane). After stirring for 10min the reverse microemulsion was formed giving a clear solution. The reaction mixture was degassed by freeze-vacuum-thaw cycles (four cycles) and kept under Argon atmosphere. 60uL of 25% (w/w) ammonium persulfate solution and 40uL of TEMED were added to initiate the polymerization. The reaction was stirred at room temperature for 3 h, and reaction mixture was monitored by <sup>1</sup>H NMR to ensure the completion of polymerization. The reaction mixture was precipitated by ethanol, and then filtered and washed using an Amicon ultra-filtration cell (Millipore Corp., Bedford, MA). The NPs were dispersed into MilliQ water using ultrasonic treatment and was dialyzed against MilliQ water for 4 days.

#### **Preparation of pH nanosensor NP-Oregon-Fluorescein-RhB**

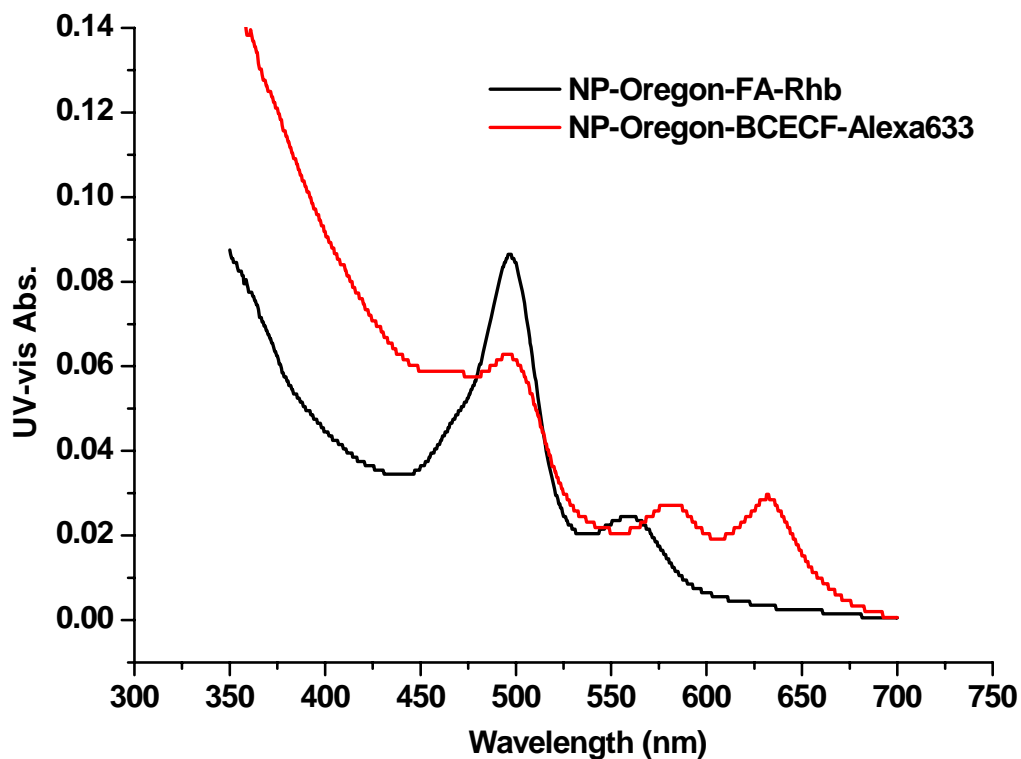
To 50mL NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> buffer (pH 9.5) solution was added 1mL of NPs-NH<sub>2</sub> (50mg/mL in MilliQ water). 38uL of FITC (1mg/mL in dry DMSO), 45uL of Oregon Green 488 isothiocyanate (1mg/mL in dry DMSO) and 100uL of RhB ITC (1mg/mL in dry DMSO) were added to the NPs-NH<sub>2</sub> solution. The final pH value of reaction mixture was pH 9.2. After 1h, the reaction mixture was transferred to dialysis tube (cutoff pore size 300KDa) and dialyzed against milliQ water for 2 days. The water was exchanged 4 times during this period.

#### **Preparation of pH nanosensor NP-Oregon-BCECF-Alexa633**

To 50mL NaHCO<sub>3</sub>/Na<sub>2</sub>CO<sub>3</sub> buffer (pH 9.5) was added 1mL of NPs-NH<sub>2</sub> (50mg/mL in MilliQ water). 10uL of BCECF (1mg/mL in dry DMSO), 10uL of Oregon Green 488 isothiocyanate (1mg/mL in dry DMSO) and 20uL of Alexa 633 succinimidyl ester (1mg/mL in dry DMSO) were added to the NPs-NH<sub>2</sub> solution. The final pH value of the

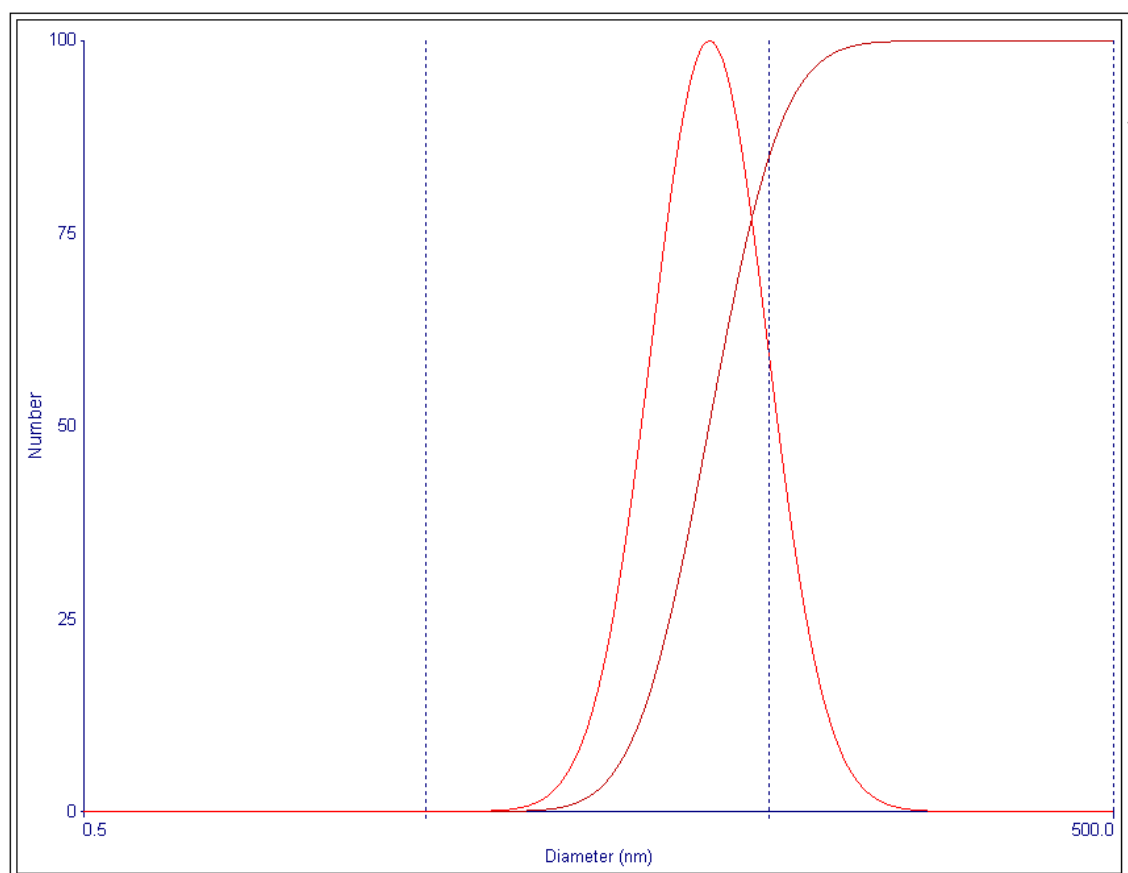
reaction mixture was pH 9.2. After 1h, the reaction mixture was transferred to dialysis tube (cutoff pore size 300KDa) and dialyzed against milliQ water for 2 days. The water was exchanged 4 times during this period.

### UV-vis spectra of nanosensors



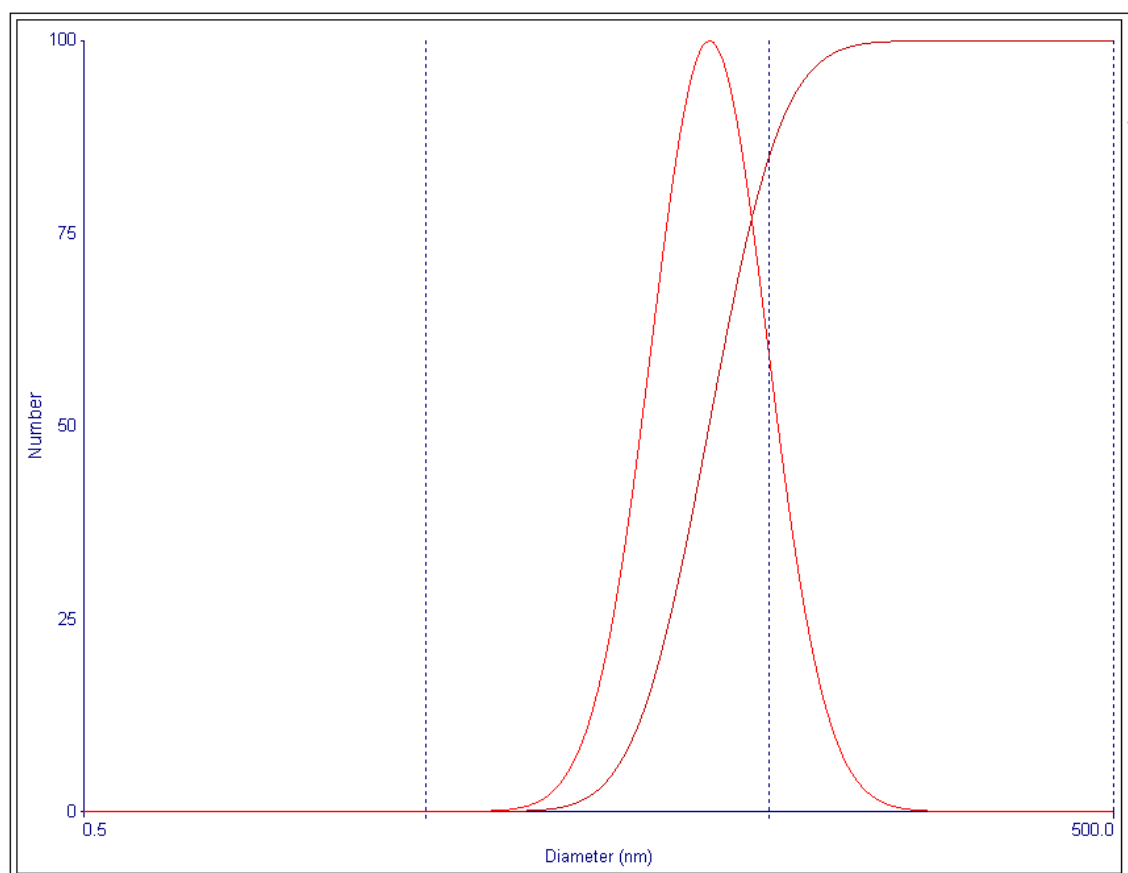
**Figure S1.** UV-vis spectra of nanosensors NP-Oregon-Fluorescein (FA)-Rhb and NP-Oregon-BCECF-Alexa633.

## DLS spectra



**Figure S2.** DLS spectrum of NP-Oregon-Fluorescein-RhB.

## DLS spectra



**Figure S3.** DLS spectrum of NP-Oregon-BCECF-Alexa633.

