Supportive Information

Quantum Dot FRET-Based Probes in Thin Films Grown in Microfluidic Channels

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Experimental Section

Materials and methods - Poly (sodium 4-styrenesulfonate) was purchased from Scientific Polymer Products. Sodium chloride was obtained from J. T. Baker. Poly (allylamine hydrochloride) (MW ~70 000), cadmium oxide, lauric acid, trioctylphosphine oxide (TOPO), hexadecylamine (HAD), lauric acid, chloroform, diethylzinc, trioctylphosphine (TOP), methanol and selenium powder, were purchased from Sigma Aldrich. Thioglycolic acid and N-(3-Dimethylaminopropyl)-N'-ethylcarbodiimide; N-Hydroxysulfosuccinimide sodium salt were purchased from Fluka. Phosphate buffered saline was purchased from Gibco. Neurotensin was purchased from American Peptide Company. PDMS, silicone elastomer kit was purchased from Dow Corning Corporation. Dialysis cassettes with 2,000 and 3500 MWCO were purchased from Pierce. Rhodamine Red ^{TM} –X succinimidyl ester was purchased from Invitrogen. Water was purified using a UV ultra pure water purification system from Barnstead. Polydimethylsiloxane (PDMS) films were oxidized using a plasma etcher (Anatech LTD-Plasma Series). Photolithographic masks were fabricated at NIST using standard microlithography protocols. Atomic force microscopic measurements were performed using a Dimension 5000 AFM microscope from Digital Instruments.

Digital Fluorescence Microscopy – Luminescence images were obtained using a digital luminescence imaging microscopy system. The system consisted of an inverted fluorescence microscope (Olympus IX 70) equipped with a 100 W Hg lamp as a light source. The fluorescence images were collected via a 20X microscope objective with NA = 0.4. A filter cube containing a 450 ±10 nm band-pass excitation filter, a 470 nm

dichroic mirror, and a 500 nm long pass emission filter was used to ensure spectral imaging purity. A high performance 16 bit resolution, back illuminated CCD camera of Roper Scientific was used for digital imaging. Luminescence spectra of quantum dots were obtained using the same microscopy system by passing the collected luminescence signal through a 250 mm Acton spectrograph. The spectra were obtained with a high performance 16 bit resolution, back illuminated CCD camera (Roper Scientific). The Roper Scientific software WinSpec 32 was used for the acquisition of digital images and spectra. The exposure time was 0.1 seconds.

Synthesis of Luminescent Quantum Dots - CdSe/ZnS quantum dots were prepared following a method previously reported by Peng and coworkers[1] with slight modifications[2]. It involves dissolving 12.7 mg CdO in 160 mg lauric acid under nitrogen at T >200° in a three neck bottle flask. After cadmium oxide is fully dissolved, 1.94 g trioctyl phosphine oxide (TOPO) and 1.94 g hexa decyl amine (HAD) are added to the mixture and the temperature is raised to 250-300K. The heat mantles are removed and 80 mg of selenium powder in 2mL of trioctyl phosphione (TOP) is injected into the reaction. After the reaction temperature is decreased to ~200 °C, the ZnS coating is formed by injecting into the reaction mixture 2 ml TOP solution containing 250 µl hexamethyldisilathiane ($(TMS)_2S$) and 1 ml diethylzinc ($Zn(Et)_2$) .The reaction mixture is kept at 180°C for one hour and then cooled to room temperature. The resulting CdSe/ZnS quantum dots are washed three times with methanol and chloroform. The hydrophobic quantum dots turn hydrophilic and water miscible through a ligand exchange reaction of TOPO with mercapto acetic acid[3]. 2ml of TOPO coated quantum

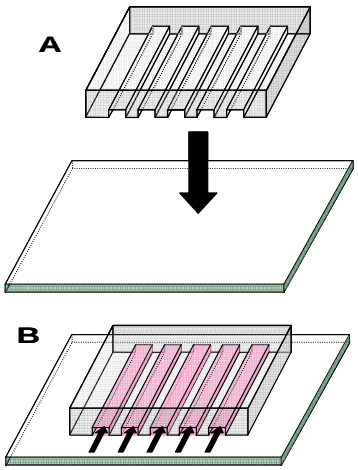
dots are suspended in 3mL of chloroform and incubated with 2ml of thioglycolic acid overnight under continuous stirring. The resulting water soluble quantum dots are extracted in 10 ml water and washed three times with water and chloroform.

Synthesis of PAH-Neurotensin-Rhodamine conjugate – 2 μM rhodamine Red^{TM} –X succinimidyl ester were reacted with 1mg/ml neurotensin at pH 7.4 in a 2 ml total reaction volume. The reaction volume was incubated for 2 hours at room temperature under continuous stirring. The unreacted rhodamine was separated out of the solution using a dialysis cassette with a 2000 MWCO cutoff filter. The remaining rhodamine labeled neurotensin solution was added to a 1 ml solution containing 1mg/ml PAH, 20 μM EDC and 50 μM sulfo NHS. The reaction mixture was incubated for 4 hours at room temperature under continuous stirring. The labeled PAH was separated from the reaction by-products by using a dialysis cassette with a 3500 MWCO cutoff filter. It should be noted that the separation of unbound rhodamine and rhodamine-labeled neurotensin as well as the separation of PAH-neurotensinn-rhodamine from smaller molecular by products could be realized as effectively by using other commercially available means like ultra centifugal filtering devices and size exclusion chromatography columns.

Fabrication of microfluidic channels on PDMS surface - Glass masters containing the channels geometry (parallel lines, 50 μm wide, 30 μm deep, 3 cm long) were fabricated following a procedure described by Martynova et al[4]. A PDMS mask was fabricated by first using a Sylgard 184 silicon elastomer kit to prepare a PDMS solution. The kit contains a silicon elastomer base (liquid resin) precursor and a silicon curing agent (cross

linking reagent). The two components were mixed in a 10:1 ratio and degassed for 45 minutes under vacuum. The PDMS solution was then poured to fill the channels of the glass master and then was cured at 100 °C for 1 h to form the PDMS mask. The PDMS mask was peeled off the glass master and was placed on an oxidized PDMS-treated glass slide (25 x 75 mm, thickness 0.93-1.05 mm pre-cleaned with sulfuric acid) to form microfluidic channels of 50 μm wide, 30 μm deep and 3 cm long (scheme S1 step A). The glass slides were coated with PDMS since PDMS was previously shown to promote polyelectrolyte deposition through surface oxidation[5]. The flat PDMS substrate was first oxidized in an O_2 (approximately 2.6 Pa) plasma for 120 seconds and then covered with the molded PDMS. This was followed by alternate deposition of polyelectrolytes to form the PEM film in the microfluidic channels following a procedure previously published by Reyes et al.[5] (scheme S1 step B).

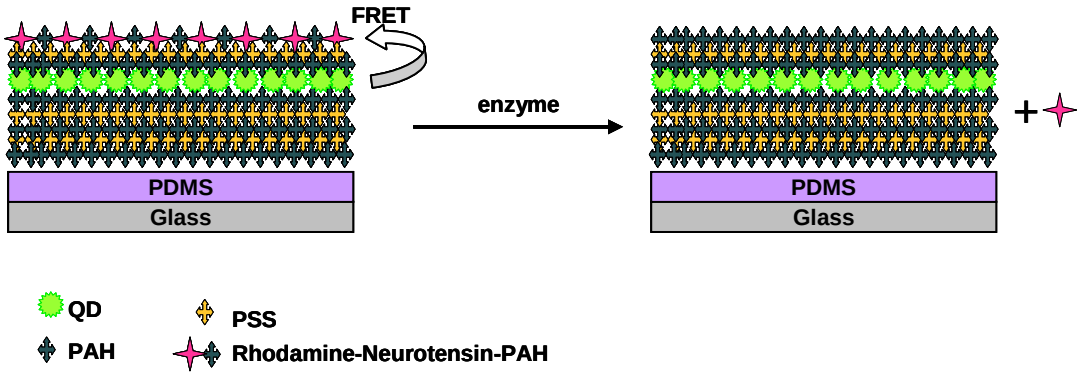
Scheme S1



Preparation of the FRET sensing film using the layer by layer (LbL) deposition method - . Scheme S2 illustrates the fabrication of PEMs in microfluidic channels by

alternate deposition of positively charged polyallylamine hydrochloride (PAH) and negatively charged polystyrene sulfonate (PSS). PAH and PSS solutions were prepared by dissolving 1 mg/ml PAH or 1mg/ml PSS in a solution of 0.1M NaCl in deionized water. The pH of the PAH and PSS solutions was adjusted to 5 and 6, respectively. The micro channels were first filled with PAH using a vacuum pump and allowed to incubate (no flow) for 30 min. The channels were then rinsed with water and dried. Five alternating layers of PSS and PAH were deposited to form the polyelectrolyte multilayer (PEM) film. Each layer was deposited by filling the channels with a PAH or PSS polyelectrolyte solution and incubating it for 10 minutes before flashing the solution out of the channels. Then, quantum dots were deposited on the PAH surface layer. The negatively charged quantum dots were then coated with another PAH layer, which was followed by the deposition of a PSS layer. Rhodamine-neurotensin-PAH was then deposited on the film surface.

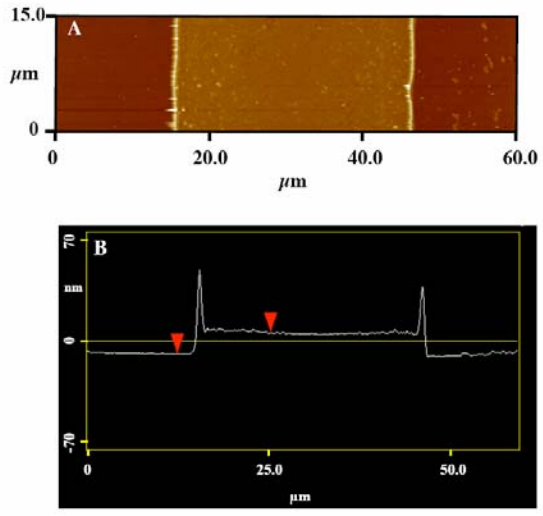
Scheme S2



Atomic Force Microscopy (AFM) - Atomic force microscopy measurements were carried out using a Dimension 5000 AFM microscope (Digital Instruments, Santa

Barbara, CA). AFM measurements were acquired in tapping mode, to obtain topographical (height) data of the deposited PEMs following Reyes *et al*[5]. Heights were measured in three different cross-sectional areas of the lines for a number of layers. All AFM measurements were made on dried PEMs channels. Figure S1A represents a top view image of a 9-layer PEM film (the yellow area) on a PDMS substrate. The left and right brown areas show the bare PDMS substrate. Figure S1B shows a cross-section (average) graph of the image shown in S1A. The average thickness of 9 layer PEM film was 14.2± 0.6 nm. Based on these AFM measurements it was concluded that the thickness of a single PEM layer is about 1.5nm.

Figure S1 –Atomic force microscopy (AFM) of 9 layers PEMs on PDMS substrate.



References

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