

Supporting Information

Detection of cancer biomarker protein on modified cellulose paper by fluorescence using aptamer-linked quantum dots

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Experimental section:

Materials. Green emitting hydrophobic oleic acid capped CdS_xSe_{1-x}/ZnS core/shell quantum dots (QDs) were from Cytodiagnostics (Burlington, ON, Canada). Whatman® cellulose chromatography papers (Grade 1, 20 cm × 20 cm), reduced L-glutathione, tetramethylammonium hydroxide solution, tris(2-carboxyethyl)phosphine hydrochloride, sodium (meta)periodate, lithium chloride, 1-(3-aminopropyl)imidazole, sodium cyanoborohydride, sodium dodecyl sulfate, albumin from bovine serum, streptavidin from streptomyces avidinii, thrombin from human plasma and bovine serum were from Sigma-Aldrich (Okaville, ON, Canada). EpCAM recombinant human protein (hIgG1-Fc Tag) was from Thermo Fisher Scientific (Canada). The aptamer 5'-DTPA-TTTTTTCACTACAGAGGTTGCGTCTGTCCCACGTTGTCATGGGGGGTGGCCTG-3' was from ACGT Corporation (Toronto, ON, Canada) and Cy3 labeled complementary DNA 5'-GTGGGACAGACGCAA-Cy3-3' was from Integrated DNA Technologies (Coralville, IA, USA).

Synthesis of water soluble glutathione-capped quantum dots (QDs-GSH). Hydrophobic oleic acid capped CdS_xSe_{1-x}/ZnS core/shell quantum dots (QDs) were transformed to be water soluble through ligand exchange with glutathione (GSH). In brief, 200 mg GSH was dissolved in 600 µL tetramethylammonium hydroxide solution. Then a chloroform solution of QDs prepared by adding 75 µL of 10 µM hydrophobic QDs in 2 mL chloroform was mixed with GSH solution. The mixture was allowed to stand overnight, and QDs-GSH were collected by centrifugation at 9000 rpm for 5 minutes. Next, QDs-GSH were purified three times by dissolving in 50 mM borate buffer (pH 9.25) containing 100 mM NaCl, and precipitation by adding ethanol, followed by collection using centrifugation. Finally, purified QDs-GSH were dissolved in 50 mM borate buffer (pH 9.25) and stored at 2-8 °C up to 6 months.

Synthesis of aptamer-linked quantum dots (QDs-Apt). The aptamer modified with dithiol phosphoramidite (DTPA) was conjugated to the surface of water-soluble QDs-GSH through self-assembly by *in situ* reduction of disulfide to dithiol of DTPA by tris(2-carboxyethyl)phosphine hydrochloride (TCEP). Briefly, 2 nmol DTPA modified aptamer in borate buffer saline pH 9.25 (50 mM, 100 mM NaCl) was incubated with 500 times molar excess of TCEP solution for 15 minutes. Then 0.2 nmol QDs-GSH in 50 mM borate buffer solution pH 9.25 and 5 μ L of 1 M NaOH were added to the aptamer solutions. The final volume of solutions was adjusted to 495 μ L by adding borate buffer saline pH 9.25. The solution mixtures were agitated overnight using a vortex mixer. Subsequently, 40 μ L of 50 mM TCEP solution in borate buffer pH 9.25 was added and 100 μ L of 2.5 M NaCl solutions was then slowly added (15 μ L in 15 minutes interval), followed by agitation overnight using a vortex mixer. The aptamer-linked quantum dots were purified to remove free aptamer using a 100 kDa centrifugal filter. The final solutions were stored at 2-8 °C up to 2-3 months.

Modification of the cellulose paper. Paper was modified with imidazole groups to immobilize the quantum dots on the cellulose. Circular reaction zones of diameter 3 mm were defined on cellulose chromatography paper using AutoCAD software and wax printing. Then the paper was placed in the oven to melt the wax at 120 °C for 4-5 minutes to make a hydrophobic barrier around the hydrophilic reaction zones. The hydroxyl groups of cellulose paper were transformed into aldehyde groups through periodate oxidation of cellulose at elevated temperatures using LiCl as an activator. Thus each hydrophilic reaction zone has treated with 5 μ L aqueous solution of NaIO₄ (10 mg/mL) and LiCl (30 mg/mL) and placed in an oven at 50 °C for 20 minutes. A similar oxidation procedure was repeated. Then the paper was washed with Milli-Q water and stored in a desiccator for overnight. The aldehyde groups on the cellulose paper were then functionalized with imidazole via imine formation followed by reduction. This made use of 2 μ L solutions of 160 mM 1-(3-aminopropyl)imidazole (API)

and 200 mM sodium cyanoborohydride in 100 mM HEPES buffer at pH 8.0. The solutions were added to each zone and allowed to stand for 1 hour at room temperature. Then the paper was washed with borate buffer at pH 9.25 containing 0.1 wt. % sodium dodecyl sulfate, and then washed with Milli-Q water. Finally, the modified cellulose paper was dried in a desiccator overnight.

Protein detection assay on the modified cellulose paper. The surface of modified cellulose paper was blocked with bovine serum albumin via immersion of the paper into a solution of 0.1 wt. % bovine serum albumin in 100 mM tris-borate buffer of pH 7.4 for 30 minutes to reduce the nonspecific adsorption on the paper. The paper was then washed with borate buffer at pH 9.25 for 10 minutes and dried in air. After that, 3 μ L solutions of aptamer-linked quantum dots were spotted on the paper and allowed to stand for 30 minutes. The unbound quantum dots were washed away with borate buffer solution at pH 9.25 for 10 minutes, and the modified paper was dried in a desiccator. Then 3 μ L Cy3 labeled complementary DNA solution of concentration 10 times with respect to aptamer used for the synthesis of aptamer-linked quantum dots was added and the paper was kept in the dark for 30 minutes. The excess free complementary DNA was removed by washing in borate buffer saline solution at pH 9.25, and the paper samples were dried in a desiccator. Samples of 3 μ L solution of protein in aptamer binding buffer solution at pH 7.4 (50 mM Tris-HCl, 140 mM NaCl, 1 mM MgCl₂, 5 mM KCl) and 10 % bovine serum solution at various concentrations (0.1-100 nM) were spotted on reaction zones and incubated for 1 hour. Then the displaced complementary DNA was washed away with aptamer binding buffer solution and photoluminescence spectra and digital fluorescence images were collected after drying the paper.

Data analysis.

Calculation of quantum yield and Förster distance.

The quantum yield (Φ) value of aptamer-linked green emitting quantum dots (QDs-Apt) was measured via our reported method.²⁶ The Φ value of QDs-GSH in 50 mM borate buffer was estimated using 0.1 M NaOH solution of fluorescein dye as a reference. The Φ value of fluorescein dye was reported to be 0.92. The QDs-Apt and fluorescein were excited at 406 nm and 470 nm, respectively. The Φ value of QDs-Apt was calculated using the following equation.

$$\Phi_s = \frac{OD_r}{OD_s} \times \frac{I_s}{I_r} \times \frac{n_s^2}{n_r^2} \times \Phi_r$$

Where subscripts s and r refer to the sample and reference, respectively. OD, I and n are the absorbance at the excitation wavelength, integrated fluorescence intensity and refractive index of the solvent, respectively.

The Φ value of QDs-Apt was determined to be 54 %.

Förster distance was determined via solution based measurement using the following equation.

$$R_0^6 = 8.79 \times 10^{-28} \times (n^{-4} k^2 \Phi_D J)$$

Where R_0 , n , k^2 , Φ_D and J are the Förster distance, the refractive index of the medium (a value of 1.33 was used), the orientation constant (a value of 2/3 was used), the quantum yield of the donor and the spectral overlap, respectively.

The value of J was calculated using the following formula.

$$J = \frac{\int F_D(\lambda) \varepsilon_A(\lambda) \lambda^4 d\lambda}{\int F_D(\lambda) d\lambda}$$

Where F_D and ε_A are the fluorescence intensity of the donor and the molar extinction coefficient of the acceptor as function of wavelength, λ .

The J value for the QDs-Apt/Cy3-cDNA FRET pair was determined to be $7.8 \times 10^{-10} \text{ cm}^6\text{mol}^{-1}$. Thus the Förster distance was calculated to be 6.5 nm.

Calculation of FRET and R/G ratio.

The photoluminescence (PL) spectra were background corrected and normalized with respect to the maximum emission of quantum dots. The quantitative measurement of protein was performed by calculation of FRET ratio using the following equation.

$$FRET \text{ ratio} = \left(\frac{\sum_{\lambda=560}^{590} PL(\lambda)}{\sum_{\lambda=510}^{540} PL(\lambda)} \right)_{DA} - \left(\frac{\sum_{\lambda=560}^{590} PL(\lambda)}{\sum_{\lambda=510}^{540} PL(\lambda)} \right)_D$$

Where the numerator of each term corresponds to the total PL intensity of acceptor (Cy3) in the range of 560 to 590 nm and the denominator of each term corresponds to the total PL intensity of donor (QDs) in the range of 510 to 540 nm. The subscript DA and D represents PL measurement was performed in the presence of donor and acceptor together and in the absence of acceptor respectively.

The captured digital fluorescence images of reaction zones under UV lamp by the mobile camera were split into red (R), green (G) and blue (B) channels by using ImageJ software.¹⁷ Then the fluorescence intensity (I) of R and G channels was measured and the R/G ratio was calculated using the following equation.

$$\frac{R}{G} \text{ ratio} = \left(\frac{I_R}{I_G} \right)_{DA} - \left(\frac{I_R}{I_G} \right)_D$$

Where I_R and I_G correspond to the fluorescence intensity of the R and G channel respectively. The subscript DA and D represents measurement was performed in the presence of donor and acceptor together and in the absence of acceptor respectively.

Instrumentation.

Solution-phase UV-visible absorption and photoluminescence spectra were collected using HP8452A Diode-Array Spectrophotometer (Hewlett Packard Corporation, Palo Alto, CA) and QuantaMaster Photon Technology International spectrofluorimeter (London, ON, Canada) equipped with a xenon arc lamp (Ushio, Cypress, CA) as an excitation source and a red-sensitive R928P photomultiplier tube as a detector (Hamamatsu, Bridgewater, NJ), respectively. The hydrodynamic sizes and zeta potentials were measured by dynamic light scattering using Malvern Zetasizer Nano ZS instrument (Malvern Instruments Ltd., Worcestershire, UK). Photoluminescence spectra from the reaction zones on cellulose paper were collected using excitation at 402 nm from a diode laser (25 mW) using a Nikon Eclipse L150 epifluorescence microscope (Nikon, Mississauga, ON). The digital fluorescence images of the reaction zones on cellulose paper were captured under UV light (365 nm) by Moto G3 mobile camera.

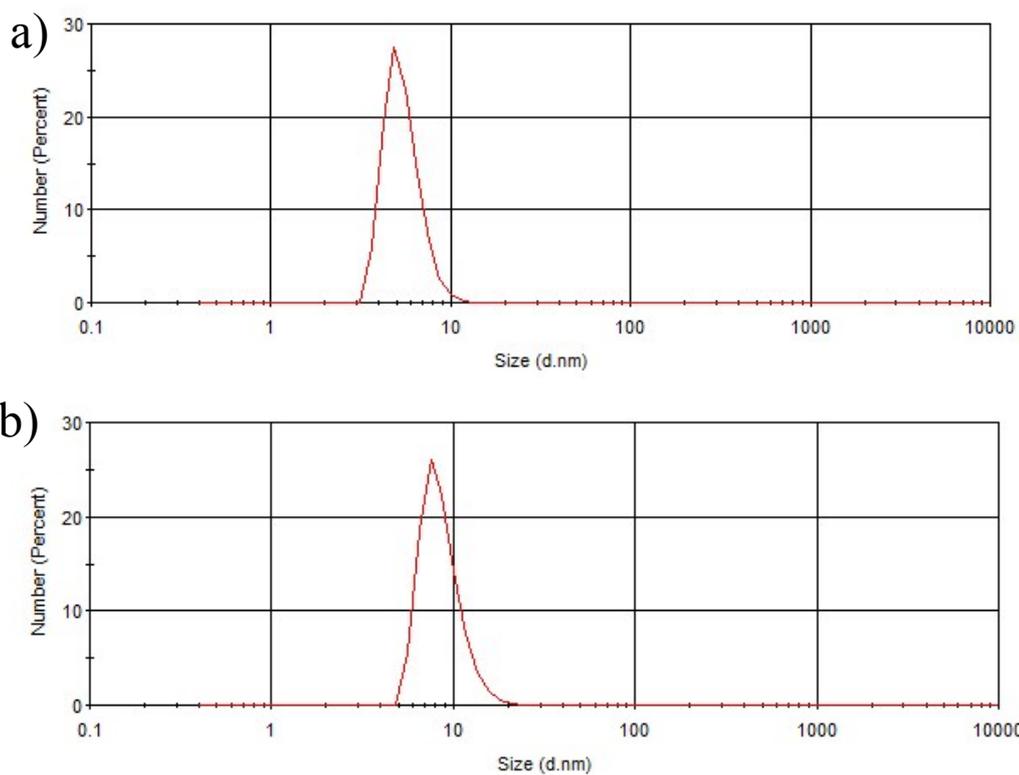


Fig. S1 The hydrodynamic size distribution of glutathione-capped quantum dots (QDs-GSH) and aptamer-linked quantum dots (QDs-Apt). The hydrodynamic size of QDs-GSH and QDs-Apt in water were 5.4 nm and 8.6 nm, respectively.

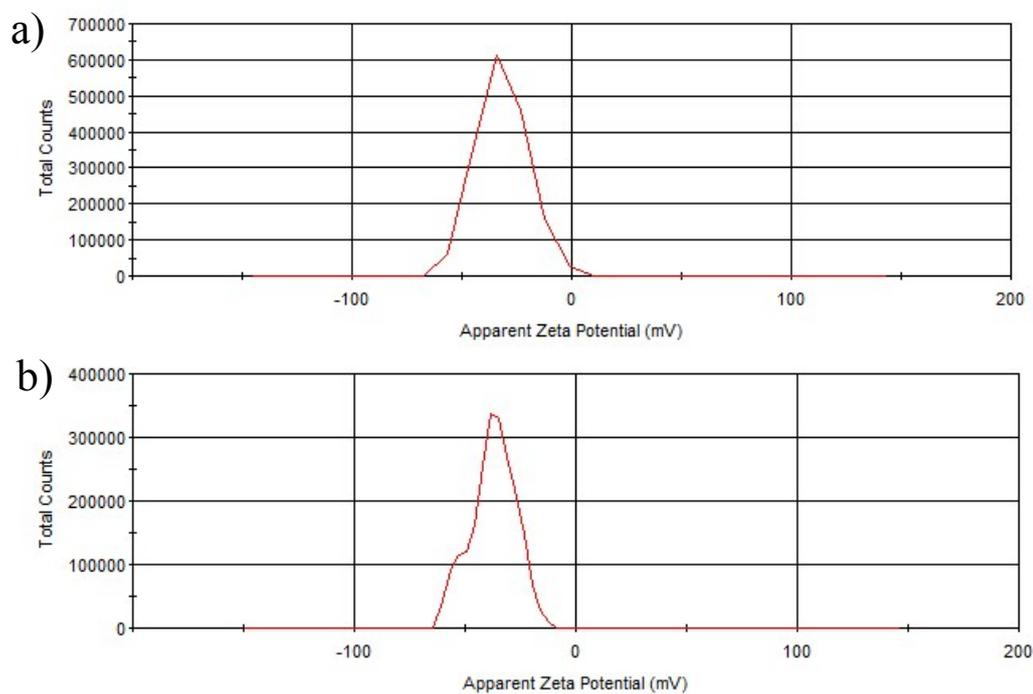


Fig. S2 Zeta potential distribution of glutathione-capped quantum dots (QDs-GSH) and aptamer-linked quantum dots (QDs-Apt) in borate buffer at pH 9.25. The zeta potential value of QDs-GSH and QDs-Apt were -31.6 mV and -37.3 mV, respectively.

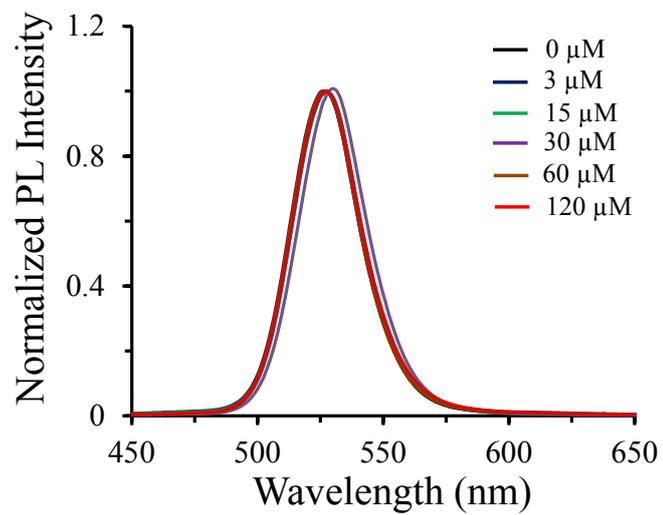


Fig. S3 Normalized photoluminescence spectra of glutathione-capped quantum dots (QDs-GSH) on modified cellulose paper in presence of Cy3 labeled complementary DNA at various concentrations (0-120 μM).

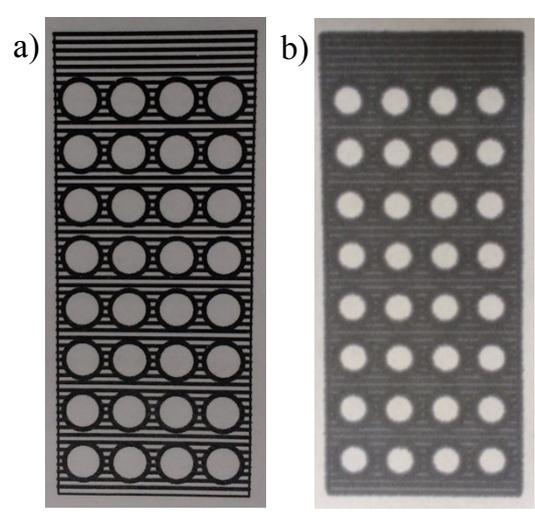


Fig. S4 Digital images of physically modified cellulose paper using AutoCAD software and wax printing a) before and b) after heating at 120 °C.

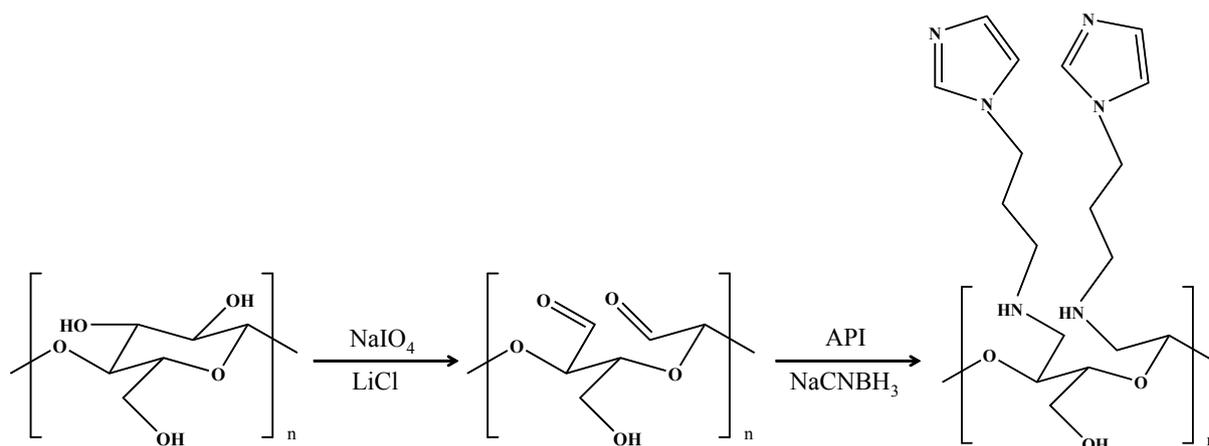


Fig. S5 The synthetic routes for chemical modification of cellulose paper to immobilize the quantum dots on the cellulose paper.

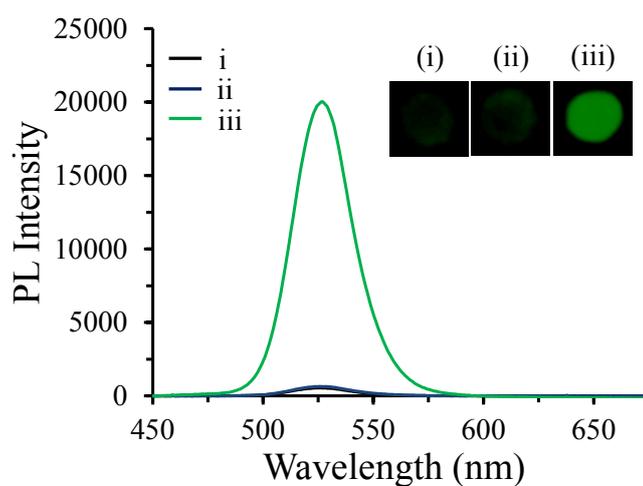


Fig. S6 Photoluminescence spectra and corresponding digital fluorescence images of QDs-GSH (200 nM) after immobilization on reaction zones of i) unmodified cellulose paper, ii) aldehyde modified cellulose paper and iii) imidazole modified cellulose paper.

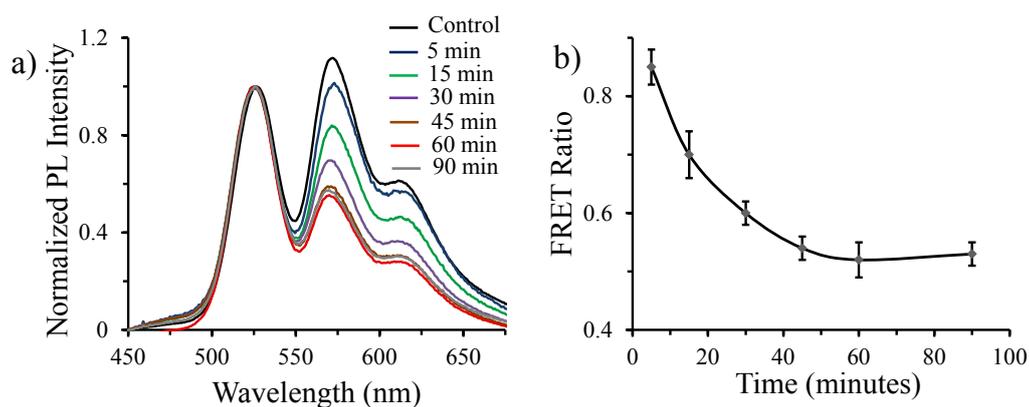


Fig. S7 a) Normalized photoluminescence spectra and b) FRET ratios calculated from normalized photoluminescence spectra of the reaction zones in absence of any analyte (control) and in presence of EpCAM (100 nM) in buffer solution at various times.

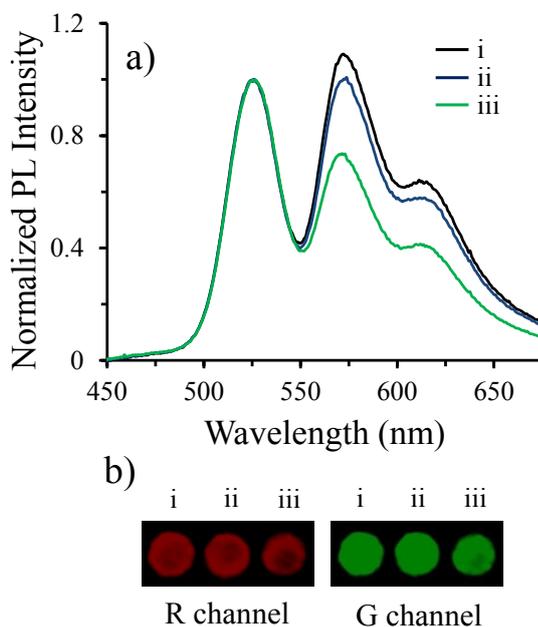


Fig. S8 a) Normalized photoluminescence spectra and b) red (R) and green (G) channels of digital fluorescence images of the reaction zones in absence of any analyte (i), in presence mixture of proteins without EpCAM (ii) and in presence mixture of proteins with EpCAM (iii). The final concentration of each protein in buffer solution is 100 nM.

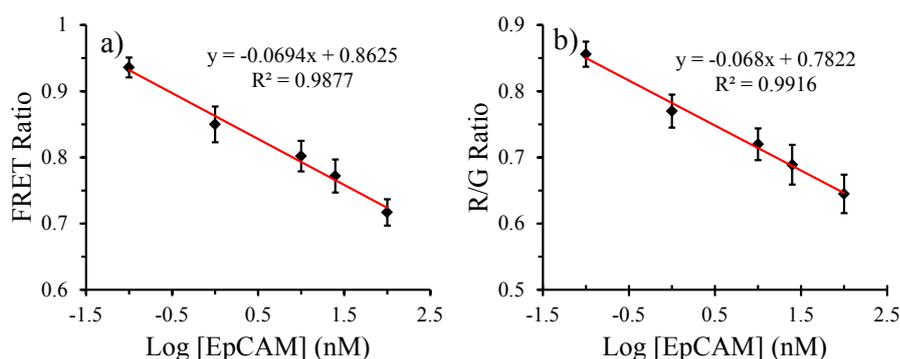


Fig. S9 a) FRET ratio measured from normalized photoluminescence spectra and b) R/G ratio measured from the intensity of R and G channels of the reaction zones on modified cellulose paper in presence of EpCAM protein in 10 % bovine serum solution at various concentrations (0.1, 1, 10, 25 and 100 nM).

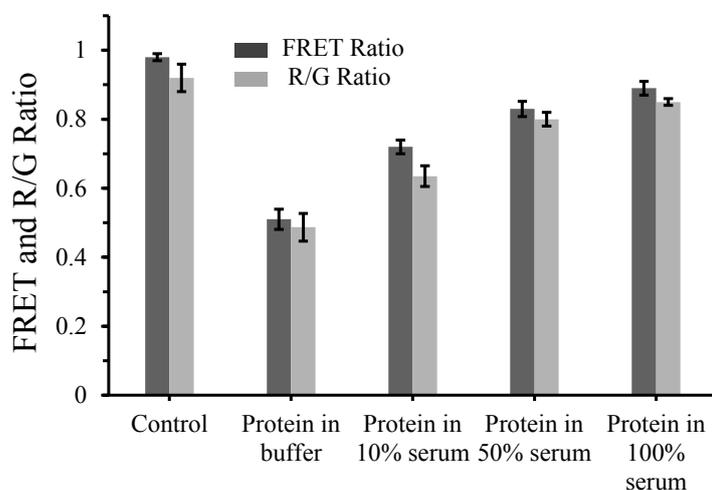


Fig. S10 FRET ratio and R/G ratio of the reaction zones in absence of any analyst (control) and in presence of EpCAM protein (100 nM) in buffer solution, 10 % bovine serum solution, 50 % bovine serum solution and 100 % bovine serum solution.

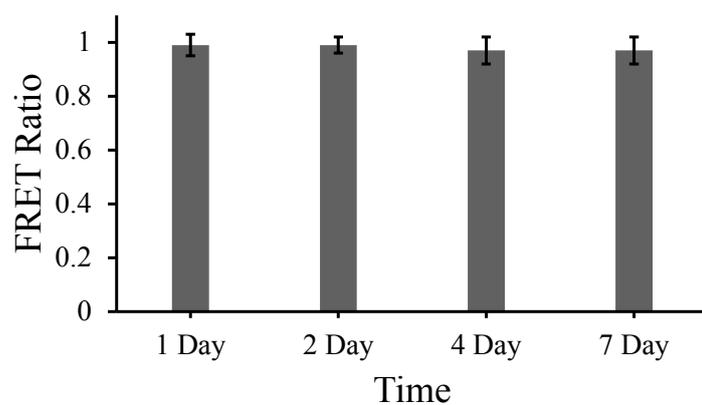


Fig. S11 FRET ratio calculated from normalized photoluminescence spectra of the control reaction zones on modified cellulose paper stored in 2-8 °C for different times.