Electronic Supplementary Material (ESI) for Journal of Materials Chemistry B.

Supporting Information

Biomolecule Detection in Porous Silicon based Microcavities via Europium Luminescence Enhancement

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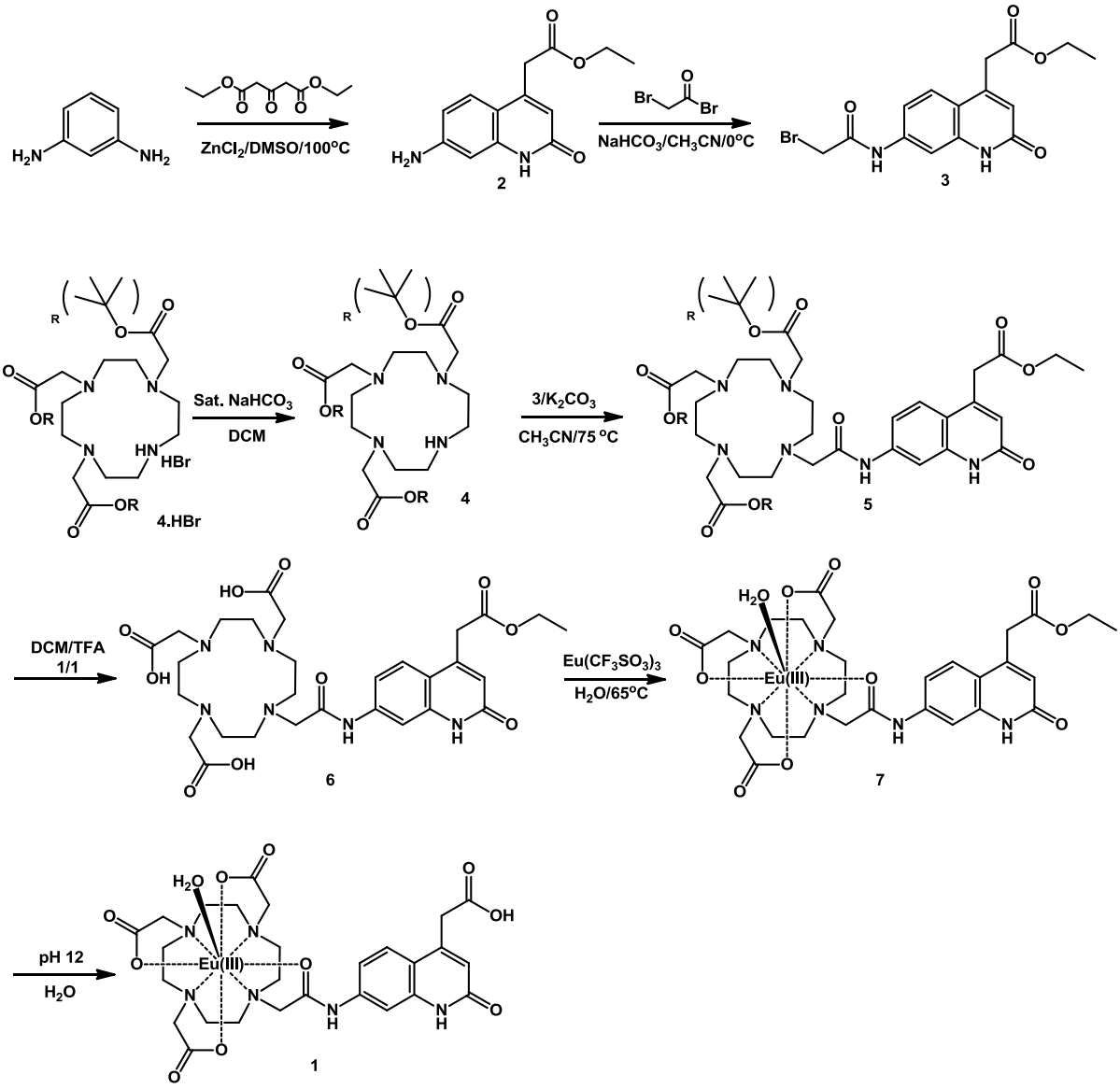
Synthesis of Eu(III) complex (1)



Synthesis of 4-Quinolineacetic acid, 7-amino-1,2-dihydro-2-oxo-, ethyl ester (2)

Diethyl-1,3-acetonedicarboxylate (4.040 g, 20 mmol) and $ZnCl_2$ (2.742 g, 20 mmol) were added to a stirred solution of 1,3-phenylenediamine (2.164 g, 20 mmol) in DMSO (5 ml). The mixture was stirred at 100 ^oC under N_2 atmosphere for 48 hours and cooled to room temperature before the addition of absolute ethanol (8 ml). The mixture was then poured into citric acid (0.1 M, 150 ml). The resulting precipitate was collected by vacuum filtration and washed with water (10 ml), diethyl ether (10 ml) and ice cold CH_3CN (10 ml) to obtain the product as a dark purple solid. Yield: 3.349 g (68%). TLC: silica $CHCl_3/C_2H_5OH$ (10/1), R_f 0.2. IR (KBr): ν = 3375 (br), 2982, 2931, 1717, 1655, 1543, 1474, 1458, 1424, 1370, 1331, 1258, 1204, 1161, 1026 cm^{-1}. ^1H NMR (DMSO-d_6, 500 MHz): δ 7.25 (d, 1H, J = 9 Hz,

Ar**H**), 6.44 (dd, 1H, *J* = 9, 2 Hz, Ar**H**), 6.38 (d, 1H, *J* = 2 Hz, Ar**H**), 6.01 (s, 1H, Ar**H**), 4.09 (q, 2H, *J* = 7 Hz, C**H**$_2$CH$_3$), 3.76 (s, 2H, C**H**$_2$CO), 1.17 (t, 3H, *J* = 7 Hz, C**H**$_3$); ^{13}C NMR (DMSO-d_6, 500 MHz): δ 170.6 (**C**=O), 162.7 (**C**=O), 151.6 (Ar**C**), 144.9 (Ar**C**), 141.5 (Ar**C**), 125.9 (Ar**C**), 116.6 (Ar**C**), 111.1 (Ar**C**), 110.1 (Ar**C**), 97.2 (Ar**C**), 61.0 (**C**H$_2$), 38.0 (**C**H$_2$), 14.5 (**C**H$_3$). ESI-MS$^+$: *m/z* = 247.2 (M+1)$^+$.

Synthesis of 4-Quinolineacetic acid, (7-(2-Bromo-acetylamino)])-1,2-dihydro-2-oxo-, ethyl ester (3)

Compound **2** (482 mg, 2.0 mmol) and NaHCO$_3$ (821 mg, 9.8 mmol) were stirred in CH$_3$CN (30 ml) at 0 ^{o}C for 20 min, bromoacetyl bromide (787 mg, 3.9 mmol) in CH$_3$CN (2ml) were added drop-wise to the above solution. The mixture was allowed to warm to room temperature and stirred overnight. The solvent was removed under reduced pressure and water was added to the residue. The resultant precipitate was collected by filtration, washed with water and dried under high vacuum to yield the product as a brown powder. Yield: 620 mg (84%). TLC: silica CHCl$_3$/C$_2$H$_5$OH (10/1), R$_f$ = 0.3. IR (KBr): ν = 3441, 3279, 2978, 2955, 1724, 1647, 1613, 1574, 1539, 1470, 1400, 1316, 1211, 1161 cm^{-1}. ^{1}H NMR (DMSO-d_6, 500 MHz): δ 11.75 (s, 1H, N**H**), 10.67 (s, 1H, N**H**), 7.78 (d, 1H, *J* = 2 Hz, Ar**H**), 7.57 (d, 1H, *J* = 8.5 Hz, Ar**H**), 7.30 (dd, 1H, *J* = 8.5, 2 Hz, Ar**H**), 6.38 (s, 1H, Ar**H**), 4.08 (m, 4H, 2 x C**H**$_2$), 3.91 (s, 2H, C**H**$_2$), 1.18 (t, 3H, *J* = 2 Hz, C**H**$_3$). ^{13}C NMR (DMSO-d_6, 125.8 MHz,): δ 169.4 (**C**=O), 164.8 (**C**=O), 161.3 (**C**=O), 143.7 (Ar**C**), 139.8 (Ar**C**), 139.1 (Ar**C**), 124.9 (Ar**C**), 120.7 (Ar**C**), 114.5 (Ar**C**), 113.0 (Ar**C**) , 104.3 (Ar**C**), 60.1 (**C**H$_2$), 36.7 (**C**H$_2$), 29.7 (**C**H$_2$), 13.6 (**C**H$_3$). ESI-MS$^+$: *m/z* = 367.0 (M+1)$^+$.

Synthesis of tert-butyl cyclen (4.HBr)

Cyclen (1.010 g, 5.9 mmol) and sodium acetate trihydrate (2.614 g, 19.2 mmol) were dissolved in DMF (25 ml) and the reaction cooled to 0 °C (ice bath). This reaction mixture was stirred for 30 minutes then tert-butyl bromoacetate (3.745 g, 19.2 mmol) in DMF (10 ml) was added dropwise over a 2 hour period whilst reaction was maintained at 0 °C. The reaction was then allowed to warm to room temperature and stirred for a further 72 hours, after which the DMF in the reaction mixture was evaporated until it became paste like. The paste was diluted with 50 ml of diethyl ether and cooled to -5 °C using an ice-ethanol bath,

and incubated for 1 hour. A white precipitate was formed and collected by filtration.The precipitate was washed with cold diethyl ether (2x25 ml) and dried. The white solid was collected and dissolved in chloroform. This solution was washed with water (2x15 ml) and a saturated solution of NaBr (1x15 ml). The organic layer was collected and dried over Na_2SO_4. The solevent was removed under reduced pressure to yield thin colorless oil. The oil was diluted with hexane (40 ml) and stirred for 30 minutes at 0 °C. The resulting white solid was collected by filtration and further dried under high vacuum to yield **4.HBr** as a white powder. Yield: 3.215 g (92%). TLC: Silica, DCM/CH_3OH (90/10), Rf = 0.6. ^{1}H NMR ($CDCl_3$, 300 MHz): δ 3.35 (s, 4H, COC**H$_2$**), 3.26 (s, 2H, COC**H$_2$**), 3.07 (br, 4H, C**H$_2$**), 2.89-2.86 (m, 12H, C**H$_2$**), 1.43 (s, 18H, C**H$_3$**), 1.42 (s, 9H, C**H$_3$**). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 170.8 (**C**=O), 169.9 (**C**=O), 82.2 (**C**), 82.0 (**C**), 58.5 (**C**H_2), 51.6 (**C**H_2), 49.5 (**C**H_2), 47.9 (**C**H_2), 28.6 (**C**H_3), 28.5(**C**H_3). ESMS$^+$: *m/z* 515.4 $(M+1)^+$.

Synthesis of 1,4,7-Tris(*tert*-butoxycarbonylmethyl)-10-(4'-Quinolineacetic acid, (7'-acetamide)-1',2'-dihydro-2'-oxo-, ethyl ester) -1,4,7,10-tetraazacyclododecane (5)

Compound **4.HBr** (179 mg, 0.30 mmol) was dissolved in DCM (20 ml) and washed with saturated $NaHCO_3$ solution (3 x 10 ml). The organic layer was dried over Na_2SO_4. The solvent was removed under reduced pressure to yield **4** which was then added to a stirred solution of **3** (204 mg, 0.56 mmol) and K_2CO_3 (258 mg, 1.87 mmol) in CH_3CN (20 ml) at 75 ^{o}C for 24 hours. The reaction mixture was filtered through celite and the solvent removed under reduced pressure. DCM (20 ml) was added to the residue and the organic layer was washed with water (3 x 20ml), dried over Na_2SO_4, and the solvent removed under reduced pressure to yield the product as a yellow oil. Yield: 220 mg (92%). IR (KBr): ν = 3426 (br), 2978, 2936, 2828, 1728, 1659, 1624, 1586, 1520, 1455, 1393, 1370, 1312, 1227, 1162, 1115, 1026 cm^{-1}. ^{1}H NMR (CD_3OD, 500 MHz): δ 7.95 (d, 1H, *J* = 9 Hz, Ar**H**), 7.56 (d, 1H, *J* = 8 Hz, Ar**H**), 7.36 (s, 1H, Ar**H**), 6.50 (s, 1H, Ar**H**), 4.2 to 2.0 (br, 28H, C**H$_2$**), 1.5-1.2 (m, 30H, C**H$_3$** and CH_2C**H$_3$**). ^{13}C NMR (CD_3OD, 125.8 MHz): δ 173.0 (**C**=O), 171.6 (**C**=O), 170.1 (**C**=O), 163.6 (br, Ar**C**), 145.9 (Ar**C**), 141.3 (Ar**C**), 139.0 (Ar**C**), 124.8 (Ar**C**), 119.8 (Ar**C**), 115.8 (Ar**C**), 115.6 (Ar**C**) , 105.3 (Ar**C**) , 81.52 (**C**$(CH_3)_3$), 81.48 (**C**$(CH_3)_3$)), 61.0 (**C**H_2), 56.5 (**C**H_2), 55.4 (m, **C**H_2), 53.5 (**C**H_2), 52.6 (br, **C**H_2), 37.9 (**C**H_2), 27.08 (**C**H_3), 27.06 (**C**H_3), 13.2 (**C**H_3). ESI-MS$^+$: *m/z* = 801.7 $(M+1)^+$.

Synthesis of 1,4,7-Tris(carbonylmethyl)-10-(4'-Quinolineacetic acid, (7'-acetamide)-1',2'-dihydro-2'-oxo-, ethyl ester)-1,4,7,10-tetraazacyclododecane (6)

Compound **5** (220 mg, 0.27 mmol) was dissolved in a DCM/TFA (1/1) solution and stirred at room temperature overnight. The solvent was removed under reduced pressure to yield a brown residue. The residue was then redissolved in water and the solution was adjusted to pH 7 using NaOH (1 M). The solvent was then removed under reduced pressure to yield the product as a hygroscopic solid and used without further purification. IR (KBr): ν = 3431 (br), 1686, 1439, 1408, 1211, 1134, 1022 cm^{-1}. ^{1}H NMR (D_2O, 500 MHz): δ 7.62 to 6.40 (m, 4H, (not resolved) Ar**H**), 4.22 to 3.14 (m, 26H, (not resolved) C$\mathbf{H_2}$), 1.26 (m, 3H, J = 7 Hz, C$\mathbf{H_3}$). ^{13}C NMR (D_2O, 125.8 MHz): δ 178.3 (**C**=O), 174.6 (**C**=O), 172.1 (**C**=O), 170.6 (m, **C**=O), 145.8 (Ar**C**), 139.6 (Ar**C**), 105.2 (Ar**C**), 62.6 (CH_2), 56.9 (CH_2), 56.2 (CH_2), 55.3 (CH_2), 51.8 (CH_2), 51.2 (CH_2), 48.0 (CH_2), 46.4 (CH_2), 37.6, (CH_2) 13.4 (CH_3). ESI-MS^+: *m/z* = 633.5 $(M+1)^+$.

Synthesis of 1,4,7-Tris(carbonylmethyl)-10-(4'-Quinolineacetic acid, (7'-acetamide)-1',2'-dihydro-2'-oxo-, ethyl ester)-1,4,7,10-tetraazacyclododecane.Eu (7)

Compound **6** (0.30 mmol) and $Eu(CF_3SO_3)_3$ (177 mg, 0.30 mmol) were dissolved in water (10 ml), the solution adjusted to pH 7.5 and stirred overnight at 65 ^{o}C. The product was then used to synthesis **1** *in situ*.

Synthesis of 1,4,7-Tris(carbonylmethyl)-10-(4'-Quinolineacetic acid, (7'-acetamide)-1',2'-dihydro-2'-oxo)-1,4,7,10-tetraazacyclododecane.Eu (1)

The above solution of **7** was adjusted to pH 12 and stirred for 2 hours. The resultant precipitate was then removed by centrifugation at 8300 g for 5 min. The remaining supernatant was then adjusted to pH 7 and the solvent removed to obtain the product as a pale yellow powder. Purified through a Sephadex G10 column in CH_3OH/H_2O (1/1) yielded the product as a yellow crystal. Yield: 120 mg (53%). M.p. > 300 ^{o}C. IR (KBr): ν =3426 (br), 1616 (br), 1397, 1258, 1207, 1180, 1134, 1084, 1034 cm^{-1}. ^{1}H NMR (D_2O, 500 MHz): δ 33.83 (d), 30.90, 29.73 (d), 12.26 (d), 10.27, 8.48 to 1.47 (m), -0.38 (m), -2.97, -3.98, -4.98, -6.05, -6.65, -7.72, -8.12, -10.42, -12.02, -13.43, -14.27, -15.82, -17.17. ESI-MS^+: *m/z* = 755.6 $(M+1)^+$.

Luminescence spectrum of Eu(III) complex (1) in aqueous solution

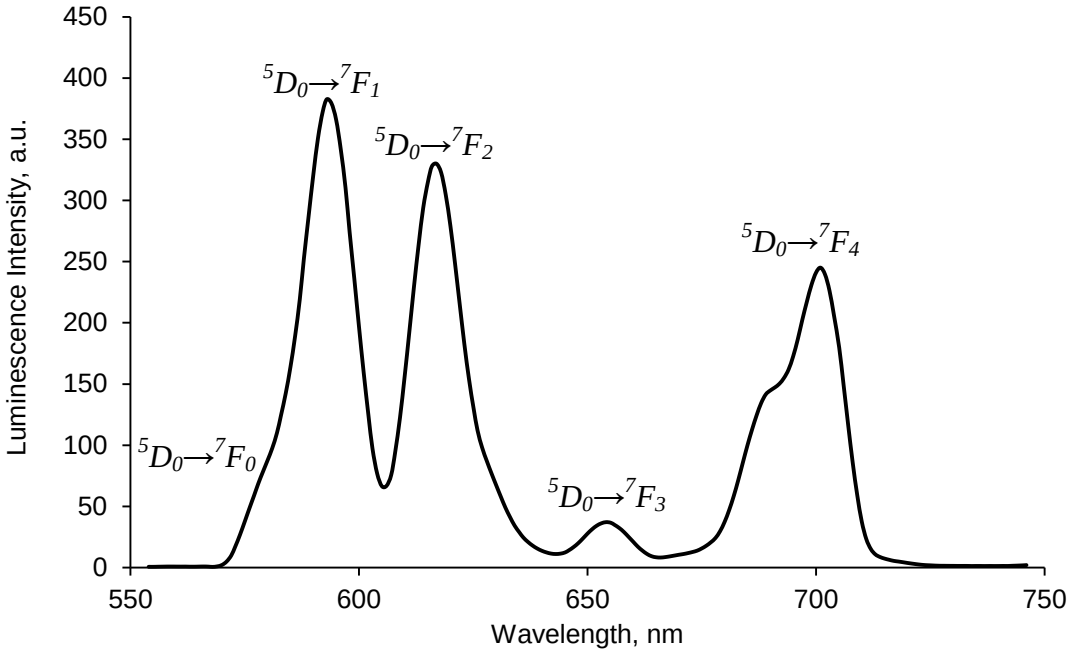


Figure S1. Luminescence spectrum of Eu(III) complex (**1**) in water. The concentration of complex **1** is $5x10^{-5}$ M.

Stability test of APTES-modified surface in ethanol

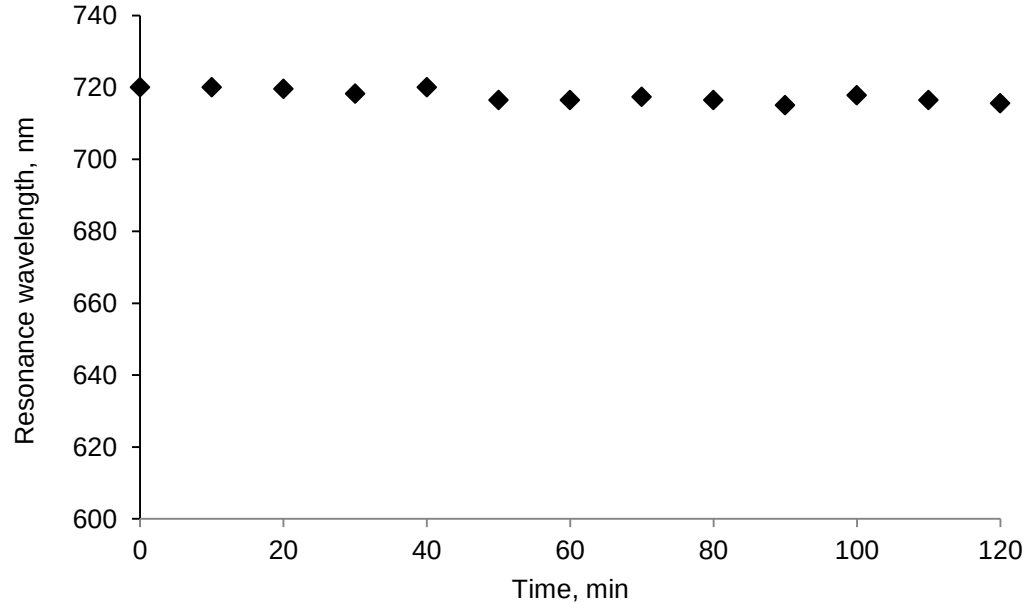


Figure S2. The resonance wavelength of the reflectance spectra of APTES modified pSiMC after incubation in ethanol for 2 h.

Optical response of MC57/23 after each surface modification

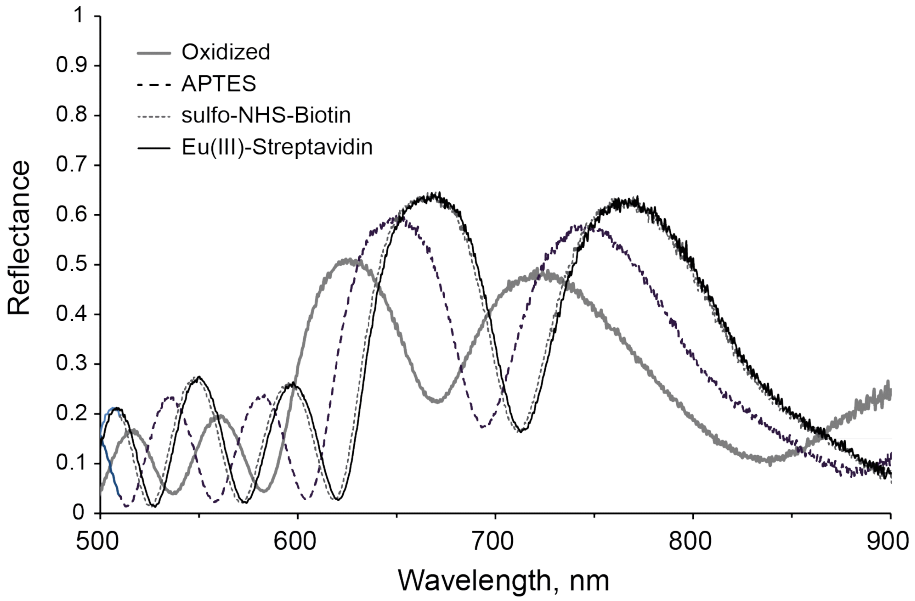


Figure S3. The evolution of the reflectance spectra of MC57/23 in air at each stage of the surface functionalization process, measured at normal incidence.

Luminescence spectrum of Eu(III) complex labeled streptavidin on MS57/23 in wound fluid

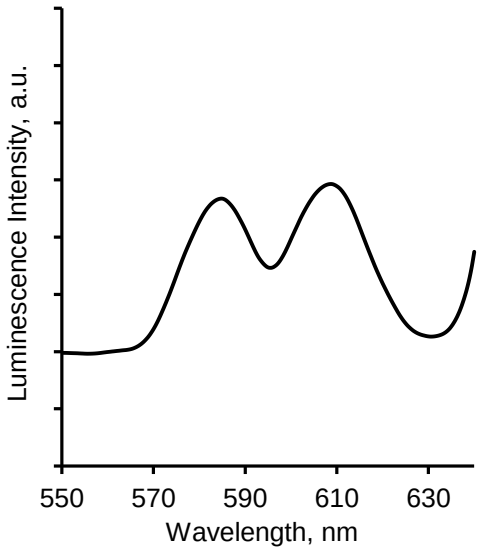


Figure S4. Luminescence spectrum of Eu(III) complex labeled streptavidin on MC57/23 in wound fluid media.

Luminescence spectrum of Eu(III) complex, 1 in H_2O at different pH

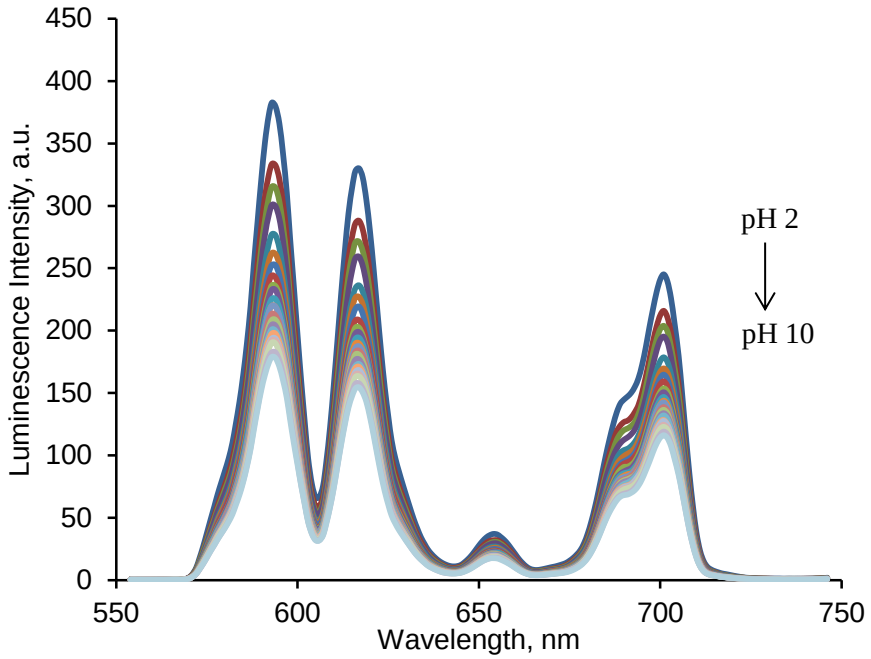


Figure S5. Luminescence spectra of Eu(III) complex, **1** in H_2O at different pH. The luminescence intensity decreases as the pH increases.

Fluorescence spectrum of Cy5 labeled streptavidin on the biotin modified surface

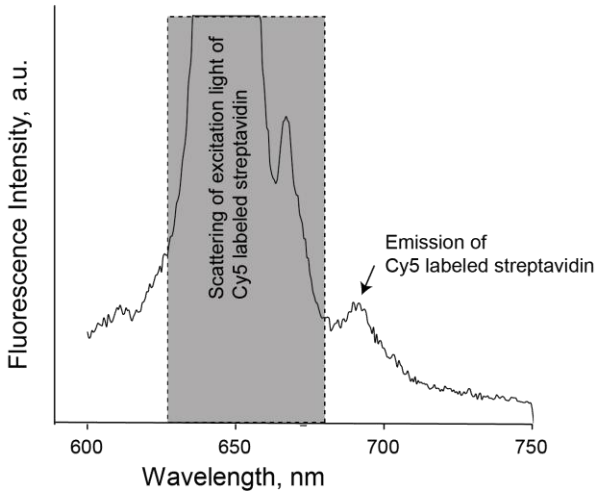


Figure S6. Fluorescence spectrum of biotin-functionalized microcavity after incubation with streptavidin labeled Cy5 dye (dashed line). Excitation/emission wavelength of the Cy5 dye were 640/670 nm.