

## **SUPPORTING INFORMATION**

### **Indo-1 derivatives for local calcium sensing**

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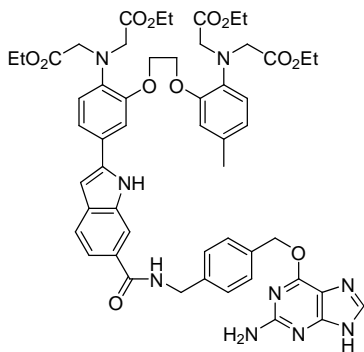
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## ORGANIC SYNTHESIS

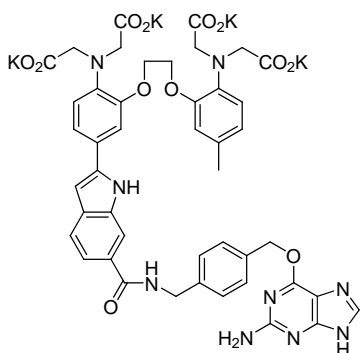
**General Procedures.** Chemicals were purchased from Sigma, Fluka, Aldrich, Acros or Invitrogen. Reverse phase preparative HPLC (RP-HPLC) was performed on a SunFire™ PrepC<sub>18</sub>OBD™ 5 μm (19 x 150 mm) column using a Waters 2777C Sample Manager, Waters 600E Controller, and Waters Fraction Collector III system. For RP-HPLC separations, a linear gradient of H<sub>2</sub>O (0.1% TFA)/MeCN (0.08% TFA) 95/5 to 0/100 and flow rate of 10 mL/min were used. Detection was achieved by a Waters 2487 Dual λ Absorbance Detector (280 nm and 350 nm). <sup>1</sup>H-NMR spectra were recorded on a Bruker ARX-400 (400 MHz) spectrometer at 298 K. Chemical shifts are reported on the δ scale in ppm from deuterated solvents, and referenced to solvent peaks (CDCl<sub>3</sub>: 7.26 ppm, CD<sub>3</sub>OD: 3.31 ppm, CD<sub>3</sub>CN: 1.94 ppm, and D<sub>2</sub>O: 4.79 ppm) as internal standards. Data are reported as follows: chemical shift, multiplicity (s = singlet, br = broad singlet, d = doublet, dd = doublet of doublets, t = triplet, q = quartet, p = quintet, and m = multiplet), integration, and coupling constants (*J* values, measured in Hz). <sup>1</sup>H-decoupled <sup>13</sup>C-NMR spectra were recorded on a Bruker ARX-400 spectrometer at 298 K. Chemical shifts are reported on the δ scale in ppm from deuterated solvents, and referenced to solvent peaks (CDCl<sub>3</sub>: 77.0 ppm, CD<sub>3</sub>OD: 49.0 ppm, and CD<sub>3</sub>CN: 1.32 ppm) as internal standards. Data are reported as follows: chemical shift and integration (if not equal to one). Mass spectra were recorded by electrospray ionization on a Micromass (Waters) ESI QqTof Ultima API spectrometer. UV spectra were measured on a Perkin Elmer Lambda10 UV/VIS spectrometer.

BG-NH<sub>2</sub> (**2**), BG-PEG<sub>4</sub>-NH<sub>2</sub> (**5**) and 5-[6-(carboxy)indol-2-yl]-5'-methyl-BAPTA ethyl ester (**1**) were prepared using published procedures (1-3).

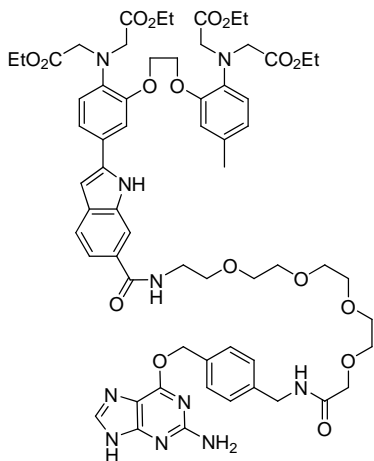
**5-[6-(*N*-(4-methyl-BG)carboxamide)indol-2-yl]-5'-methyl-BAPTA ethyl ester (3).** (BG = *O*-6-benzylguanine; BAPTA = 1,2-bis(2aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid).



EDC (8.2 mg, 42.8  $\mu\text{mol}$ ), HOBt (5.8 mg, 42.9  $\mu\text{mol}$ ) and *O*-6-(4-aminomethylbenzyl)guanine (**2**) (5.2 mg, 19.2  $\mu\text{mol}$ ) were added to a solution of 5-[6-(carboxy)indol-2-yl]-5'-methyl-BAPTA ethyl ester (**1**) (**3**) (10.0 mg, 13.1  $\mu\text{mol}$ ) in DMF (0.5 mL). The mixture was stirred overnight at room temperature. The crude product was purified by reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid and concentrated by lyophilization to yield 9.3 mg (9.2  $\mu\text{mol}$ , 70%) of BG1-Indo-1 ethyl ester (**3**).  $^1\text{H}$  NMR (400 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  7.96 (s, 1H), 7.80 (s, 1H), 7.52 (s, 2H), 7.43 (d,  $J = 8.1$ , 2H), 7.38 – 7.28 (m, 4H), 6.79 (d,  $J = 8.4$ , 1H), 6.74 (s, 1H), 6.71 – 6.61 (m, 3H), 5.47 (s, 2H), 4.58 (s, 2H), 4.25 (s, 2H), 4.15 (s, 6H), 4.06 (s, 4H), 4.02 – 3.93 (m, 8H), 2.22 (s, 3H), 1.11 – 1.02 (m, 12H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CD}_3\text{OD}$ )  $\delta$  173.6 (2C), 173.4 (2C), 171.5, 161.7, 151.7(2C), 142.6, 140.6, 140.5, 138.1, 138.0, 136.7, 133.6, 133.2, 129.7 (2C), 128.6 (2C), 128.0, 127.1, 122.5, 120.6, 119.8, 119.6, 119.5 (2C), 114.9, 112.1, 111.2, 99.2, 68.8, 68.7, 68.3, 62.2 (2C), 62.0 (2C), 54.8 (4C), 44.4, 21.1, 14.4 (4C). HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{Na}]^+$  calcd for  $\text{C}_{53}\text{H}_{59}\text{N}_9\text{O}_{12}\text{Na}$ , 1036.4181; found, 1036.4188.

**BG1-Indo-1 (4).**

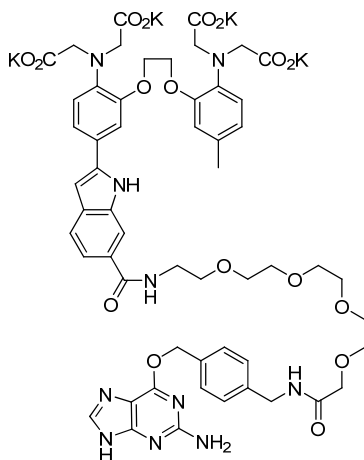
1 M KOH in an aqueous solution (50  $\mu\text{L}$ , 50  $\mu\text{mol}$ ) was added to a solution of **(3)** (7.8 mg, 7.7  $\mu\text{mol}$ ) in THF (0.5 mL). The mixture was stirred overnight at room temperature. The product precipitated during the reaction. The solvent was carefully removed using a Pasteur pipette, and the solid was washed twice with THF. The product was dried under reduced pressure and then dissolved in 10 mM MOPS buffer solution (pH 7.2). The concentration of **(4)** was estimated to be 1.49 mM by the absorption at  $\lambda = 354$  nm using the extinction coefficient of commercial Indo-1 pentapotassium salt ( $\epsilon_{346} = 33000 \text{ M}^{-1}\text{cm}^{-1}$  at pH 7.2)(4).  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.77 (s, 1H), 7.65 (s, 1H), 7.48 (d,  $J = 8.0$ , 1H), 7.36 (d,  $J = 8.0$ , 1H), 7.24 – 7.14 (m, 6H), 6.79 (br, 2H), 6.75 (d,  $J = 8.1$ , 1H), 6.69 (d,  $J = 7.9$ , 1H), 6.57 (s, 1H), 5.04 (s, 2H), 4.38 (s, 2H), 4.17 (s, 4H), 3.80 (s, 4H), 3.70 (s, 4H), 2.19 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{D}_2\text{O}$ - $\text{CD}_3\text{OD}$ )  $\delta$  180.2 (2C), 180.0 (2C), 171.2, 160.4, 159.9, 150.5, 150.2, 142.3, 141.7, 139.2, 138.6, 137.0, 136.0, 133.1, 132.9, 128.8 (2C), 128.4 (2C), 126.7, 125.0, 122.7, 120.8, 120.1, 119.4, 118.8, 118.6, 115.2, 112.1, 111.6, 98.5, 68.9, 68.6, 67.6, 58.2 (2C), 57.9 (2C), 44.2, 20.9. HRMS-ESI ( $m/z$ ):  $[\text{M}]^+$  calcd for  $\text{C}_{45}\text{H}_{39}\text{N}_9\text{O}_{12}\text{K}_4$ , 1053.1266; found, 1053.1263.

**BG2-Indo-1 ethyl ester (6).**

EDC (26.2 mg, 136.7  $\mu\text{mol}$ ), HOBt (18.5 mg, 136.9  $\mu\text{mol}$ ), and BG-PEG<sub>4</sub>-NH<sub>2</sub> (**5**) (22.6 mg, 44.9  $\mu\text{mol}$ ) were added to a solution of 5-[6-(carboxy)indol-2-yl]-5'-methyl-BAPTA ethyl ester (**1**) Error! Bookmark not defined. (33.0 mg, 43.3  $\mu\text{mol}$ ) in DMF (2.0 mL). The mixture was stirred overnight at room temperature. The crude product was purified by reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid and concentrated by lyophilization to yield 18.6 mg (14.9  $\mu\text{mol}$ , 71%) of (**6**). <sup>1</sup>H NMR (400 MHz, CD<sub>3</sub>CN)  $\delta$  7.89 (s, 1H), 7.79 (s, 1H), 7.74 (m, 2H, CONH), 7.69 (s, 1H), 7.51 (d,  $J = 8.4$ , 1H), 7.49 (s, 1H), 7.46 – 7.41 (m, 2H), 7.22 (d,  $J = 8.0$ , 2H), 7.11 (d,  $J = 8.3$ , 1H), 7.06 (d,  $J = 7.9$ , 2H), 6.96 (d,  $J = 8.1$ , 1H), 6.84 (s, 1H), 6.83 (s, 1H), 6.75 (d,  $J = 8.0$ , 1H), 5.61 (s, 2H, NH<sub>2</sub>), 5.32 (s, 2H), 4.34 (br, 2H), 4.26 (d,  $J = 5.9$ , 2H), 4.23 (br, 2H), 4.14 (s, 2H), 4.07 – 3.97 (m, 6H), 3.97 – 3.90 (m, 8H), 3.83 (s, 4H), 3.70 – 3.66 (m, 2H), 3.65 – 3.61 (m, 2H), 3.58 – 3.55 (m, 2H), 3.54 – 3.50 (m, 6H), 3.49 – 3.44 (m, 2H), 3.32 (q,  $J = 4.6$ , 2H), 2.28 (s, 3H), 1.16 (t,  $J = 7.2$ , 6H), 1.04 (t,  $J = 7.1$ , 6H). <sup>13</sup>C NMR (101 MHz, CD<sub>3</sub>CN)  $\delta$  174.1 (2C), 174.0 (2C), 172.0, 170.3,

161.1, 151.9, 151.5, 141.4, 139.6, 139.1, 137.8, 136.6, 136.4, 135.8, 132.6, 129.7 (2C), 129.2, 128.4, 128.2 (2C), 122.6, 120.5, 120.0, 119.7, 119.6, 119.5, 113.7, 112.2, 110.1, 99.7, 72.5, 71.0, 70.5 (2C), 70.1, 70.0 (2C), 69.9, 68.2, 67.4, 66.9, 62.7 (2C), 62.6 (2C), 56.0 (2C), 55.9 (2C), 43.2, 40.1, 21.1, 14.4 (2C), 14.3 (2C). HRMS-ESI ( $m/z$ ):  $[M + Na]^+$  calcd for  $C_{63}H_{78}N_{10}O_{17}Na$ , 1269.5444; found, 1269.5455.

### BG2-Indo-1 (7).

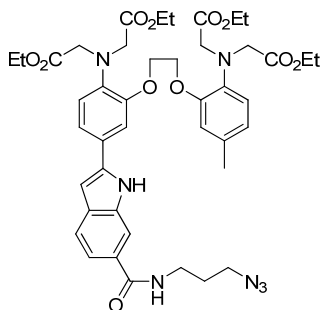


1 M KOH in an aqueous solution (45  $\mu$ L, 45  $\mu$ mol) was added to a solution of (6) (9.0 mg, 7.2  $\mu$ mol) in THF (0.5 mL). The mixture was stirred overnight at room temperature. The product precipitated during the reaction. The solvent was carefully removed using a Pasteur pipette, and the solid was washed twice with THF. The product was dried under reduced pressure and then dissolved in 10 mM MOPS buffer solution (pH 7.2). The concentration of (7) was estimated to be 1.78 mM by the absorption at  $\lambda = 356$  nm using the extinction coefficient of commercial Indo-1 pentapotassium salt ( $\epsilon_{346} = 33000 \text{ M}^{-1}\text{cm}^{-1}$  at pH 7.2) (4).  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.80 (s, 1H), 7.69 (s, 1H), 7.47 (d,  $J = 8.2$ , 1H), 7.36 (d,  $J = 7.9$ , 3H), 7.30 (s, 1H), 7.22 (d,  $J = 8.2$ , 1H),

7.17 (d,  $J = 7.8$ , 2H), 6.87 (s, 2H), 6.82 (d,  $J = 8.4$ , 1H), 6.80 – 6.71 (m, 2H), 6.54 (s, 1H), 5.26 (s, 2H), 4.29 (br, 2H), 4.28 (br, 2H), 4.23 (s, 2H), 3.84 (br, 6H), 3.73 (br, 4H), 3.57 (t,  $J = 4.5$ , 2H), 3.49 (br, 4H), 3.42 (d,  $J = 2.8$ , 2H), 3.39 (s, 4H), 3.33 – 3.31 (m, 4H), 2.21 (s, 3H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{D}_2\text{O}-\text{CD}_3\text{OD}$ )  $\delta$  180.0 (2C), 179.9 (2C), 173.2, 172.2, 160.4, 158.91, 150.8, 150.4, 142.5, 141.9, 138.7, 138.3, 137.2, 136.9, 133.4, 133.0, 129.0 (2C), 128.4 (2C), 126.9, 125.4, 122.6, 120.8, 120.2, 119.4, 118.9, 118.9, 115.0, 112.4, 111.6, 98.7, 71.3, 70.6, 70.5 (3C), 70.4 (2C), 69.9, 68.8, 68.7, 67.5, 58.3 (2C), 58.0 (2C), 43.2, 40.6, 21.0.  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{55}\text{H}_{63}\text{N}_{10}\text{O}_{17}$ , 1135.437; found, 1135.467.

**5-[6-(*N*-(3-azidopropan-1-yl)carboxamide)indol-2-yl]-5'-methyl-BAPTA ethyl ester (8).**

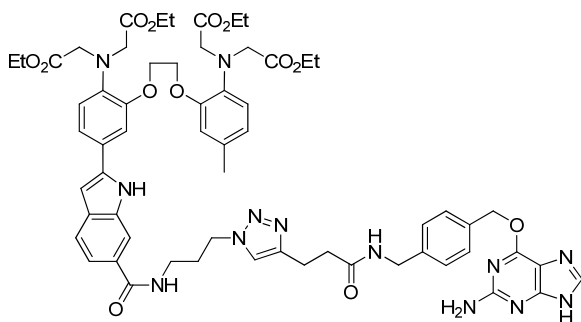
(BAPTA = 1,2-bis(2aminophenoxy)ethane-*N,N,N',N'*-tetraacetic acid).



EDC (78.0 mg, 406.9  $\mu\text{mol}$ ), HOBt (53.7 mg, 397.4  $\mu\text{mol}$ ), and 3-azido-1-propanamine (39.5 mg, 394.5  $\mu\text{mol}$ ) were added to a solution of 5-[6-(carboxy)indol-2-yl]-5'-methyl-BAPTA ethyl ester (**1**) (103.3 mg, 135.6  $\mu\text{mol}$ ) in DMF (4.0 mL). The mixture was stirred for 1 h at room temperature. The crude product was purified by reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid and concentrated by

lyophilization to yield 91.5 mg (108.4  $\mu\text{mol}$ , 80%) of (**8**).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.99 (s, 1H), 7.99 (s, 1H), 7.57 (d,  $J = 8.3$ , 1H), 7.49 (d,  $J = 8.3$ , 1H), 7.17 (d,  $J = 8.3$ , 1H), 7.12 (s, 1H), 6.97 (t,  $J = 5.3$ , 1H), 6.80 – 6.71 (m, 2H), 6.67 (d,  $J = 6.4$ , 1H), 6.66 (s, 1H), 6.56 (s, 1H), 4.14 (s, 4H), 4.11 (s, 4H), 4.10 – 4.01 (m, 12H), 3.96 (s, 2H), 3.49 (dt,  $J = 6.3, 12.3$ , 2H), 3.40 (t,  $J = 6.5$ , 2H), 2.24 (s, 3H), 1.88 (p,  $J = 6.6$ , 2H), 1.18 (t,  $J = 7.1$ , 6H), 1.12 (t,  $J = 7.1$ , 6H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  171.8 (2C), 171.4(2C), 169.5, 150.2 (2C), 141.1, 139.2, 136.4 (2C), 132.3, 132.0, 126.4, 125.8, 121.7, 119.8, 119.0, 118.8, 118.4, 118.2, 114.1, 111.2, 111.0, 98.4, 67.3, 66.7, 61.0 (2C), 60.9 (2C), 53.7 (4C), 49.4, 37.8, 28.7, 20.8, 14.0 (2C), 13.9 (2C). HRMS-ESI ( $m/z$ ):  $[\text{M} + \text{H}]^+$  calcd for  $\text{C}_{43}\text{H}_{54}\text{N}_7\text{O}_{11}$ , 844.3881; found, 844.3889.

### BG3-Indo-1 ethyl ester (**13**).

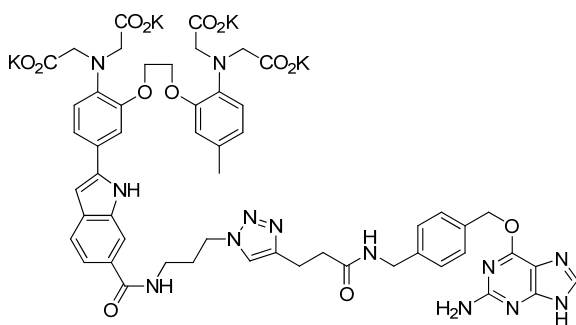


Indo-1 azido ethyl ester (**8**) (33.8 mg, 40.1  $\mu\text{mol}$ ) and BG alkyne (**2**) (18.2 mg, 51.9  $\mu\text{mol}$ ) were solubilized in  $\text{DCM}/i\text{PrOH}$  (1:1) (2.0 mL). Water (0.5 mL), an aqueous solution of 1 M  $\text{CuSO}_4$  (40  $\mu\text{L}$ , 40  $\mu\text{mol}$ ) and an aqueous solution of 2 M sodium (40  $\mu\text{L}$ , 80  $\mu\text{mol}$ ) were added to this solution. The mixture was stirred for 24 h at  $60^\circ\text{C}$ . After this period, the suspension was centrifuged to remove the solid particles. The crude product in the supernatant was purified by



reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid and concentrated by lyophilization to yield 15.3 mg (12.8  $\mu\text{mol}$ , 32%) of (**13**).  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  10.30 (s, 1H,  $N_{ind}H$ ), 7.88 (s, 2H), 7.73 – 7.62 (m, 3H), 7.40 – 7.28 (m, 2H), 7.20 – 7.02 (m, 5H), 6.96 (s, 1H), 6.95 (s, 1H), 6.87 (d,  $J = 7.7$ , 1H), 6.74 (s, 1H), 6.69 (d,  $J = 8.0$ , 1H), 6.65 (s, 1H), 6.53 (s, 1H), 5.11 (s, 2H), 4.36 (br, 2H), 4.18 – 4.09 (m, 12H), 4.09 – 3.92 (m, 10H), 3.30 (br, 2H), 2.96 (br, 2H), 2.60 (br, 2H), 2.23 (s, 3H), 2.12 (br, 2H), 1.18 – 1.05 (m, 12H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  172.8, 171.6 (2C), 171.0 (2C), 170.6, 159.6, 155.2, 150.2 (2C), 150.0, 144.0, 141.0, 138.8, 138.6, 136.2, 134.6, 134.3, 132.9, 132.1, 128.7 (2C), 127.3 (2C), 125.4 (2C), 121.8, 120.0, 119.8, 118.7 (2C), 116.9, 114.0, 113.9, 111.2, 110.0, 98.2, 67.1, 66.9, 66.8, 61.3 (2C), 61.2 (2C), 54.2 (2C), 53.7 (2C), 49.9, 43.2, 39.5, 37.0, 33.9, 29.0, 20.9, 13.9 (2C), 13.8 (2C). HRMS-ESI ( $m/z$ ):  $[M + H]^+$  calcd for  $\text{C}_{61}\text{H}_{72}\text{N}_{13}\text{O}_{13}$ , 1194.5372; found, 1194.5430.

### BG3-Indo-1 (**10**).

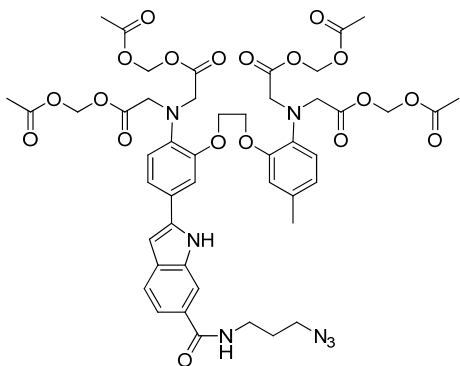


An aqueous solution of 1 M KOH (100  $\mu\text{L}$ , 100  $\mu\text{mol}$ ) was added to a solution of (**13**) (12.5 mg, 10.5  $\mu\text{mol}$ ) in THF (0.5 mL). The mixture was stirred overnight at room temperature. The solvent was removed under reduced pressure. The crude product was solubilized in water and

purified by reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid. Before lyophilization, the product fractions from HPLC were basified to pH 8-9 using 0.1 M KOH aqueous, since degradation was observed at some extension when the product was directly concentrated from TFA solutions. The product BG3-Indo-1 (**10**) was dissolved in 10 mM MOPS buffer solution (pH 7.2), and the final concentration was estimated to be 1.03 mM by the absorption at  $\lambda = 358$  nm via the extinction coefficient of commercial Indo-1 pentapotassium salt ( $\epsilon_{346} = 33000 \text{ M}^{-1}\text{cm}^{-1}$  at pH 7.2) (4).  $^1\text{H}$  NMR (400 MHz,  $\text{D}_2\text{O}$ )  $\delta$  7.66 (s, 1H), 7.53 (s, 1H), 7.53 (s, 1H), 7.45 (d,  $J = 8.2$ , 1H), 7.31 (d,  $J = 8.5$ , 1H), 7.17 (d,  $J = 7.8$ , 2H), 7.07 (d,  $J = 8.1$ , 1H), 7.00 – 6.91 (m, 3H), 6.86 – 6.78 (m, 2H), 6.74 (d,  $J = 7.8$ , 1H), 6.69 (s, 1H), 6.37 (s, 1H), 4.80 (s, 2H), 4.30 (t,  $J = 6.0$ , 2H), 4.14 (s, 2H), 4.03 (s, 4H), 3.72 (s, 4H), 3.65 (s, 4H), 3.20 (t,  $J = 5.3$ , 2H), 2.90 (t,  $J = 6.4$ , 2H), 2.46 – 2.38 (m, 2H), 2.22 (s, 3H), 2.07 – 2.01 (m, 2H). MS-ESI ( $m/z$ ):  $[\text{M}+\text{H}]^+$  calcd for  $\text{C}_{53}\text{H}_{56}\text{N}_{13}\text{O}_{13}$ , 1082.41; found, 1082.46.

**5-[6-(N-(3-azidopropan-1-yl)carboxamide)indol-2-yl]-5'-methyl-BAPTA**

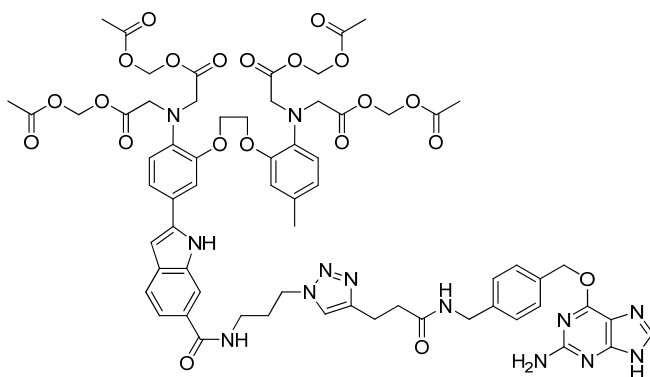
**(acetyloxy)methyl ester (11).** (BAPTA = 1,2-bis(2aminophenoxy)ethane-N,N,N',N'-tetraacetic acid).



An aqueous solution of 1 M KOH (600  $\mu$ L, 600  $\mu$ mol) was added to a solution of **(8)** (49.6 mg, 58.8  $\mu$ mol) in THF/MeOH (4:1) (2.0 mL). The mixture was stirred overnight at room temperature. After this period, 1M HCl aqueous solution (750  $\mu$ L, 750  $\mu$ mol) was added to the reaction mixture, which was then portioned between water and ethyl acetate. The organic phase was separated, washed with brine, dried over anhydrous  $\text{MgSO}_4$ , and concentrated. The crude product was dissolved in DMF (2.0 mL) and cooled to 0°C. DIPEA (100  $\mu$ L, 0.61 mmol) and bromomethyl acetate (120  $\mu$ L, 1.22 mmol) were added to this solution. The mixture was stirred for 2 h at 0°C. The crude product was purified by reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid and concentrated by lyophilization to yield 26.6 mg (26.1  $\mu$ mol, 44%) of **(11)**.  $^1\text{H}$  NMR (400 MHz,  $\text{CDCl}_3$ )  $\delta$  9.80 (s, 1H), 7.96 (s, 1H), 7.55 (d,  $J$  = 8.3, 1H), 7.42 (d,  $J$  = 7.4, 1H), 7.29 – 7.23 (m, 2H), 6.85 (d,  $J$  = 8.6, 1H), 6.77 (d,  $J$  = 8.0, 1H), 6.72 – 6.65 (m, 4H), 5.63 (s, 4H), 5.63 (s, 4H), 4.30 (s, 2H), 4.21 (s, 6H), 4.15 (s, 4H), 3.53 (q,  $J$  = 6.4, 2H), 3.40 (t,  $J$  = 6.6, 2H), 2.24 (s, 3H), 2.06 (s, 6H), 2.02 (s, 6H), 1.89 (p,  $J$  = 6.6, 2H).  $^{13}\text{C}$  NMR (101 MHz,  $\text{CDCl}_3$ )  $\delta$  170.3 (2C), 169.9 (2C), 169.7 (2C),

169.5 (2C), 168.7, 150.5, 150.4, 140.7, 138.7, 136.5, 135.9, 133.0, 131.8, 127.2, 126.6, 122.0, 119.8 (2C), 119.5, 119.3, 118.0, 114.6, 111.3, 111.0, 98.8, 79.3 (2C), 79.2 (2C), 67.5, 67.0, 53.5 (2C), 53.3 (2C), 49.4, 37.7, 28.8, 20.9, 20.6 (4C). HRMS-ESI ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{47}H_{54}N_7O_{19}$ , 1020.3474; found, 1020.3447.

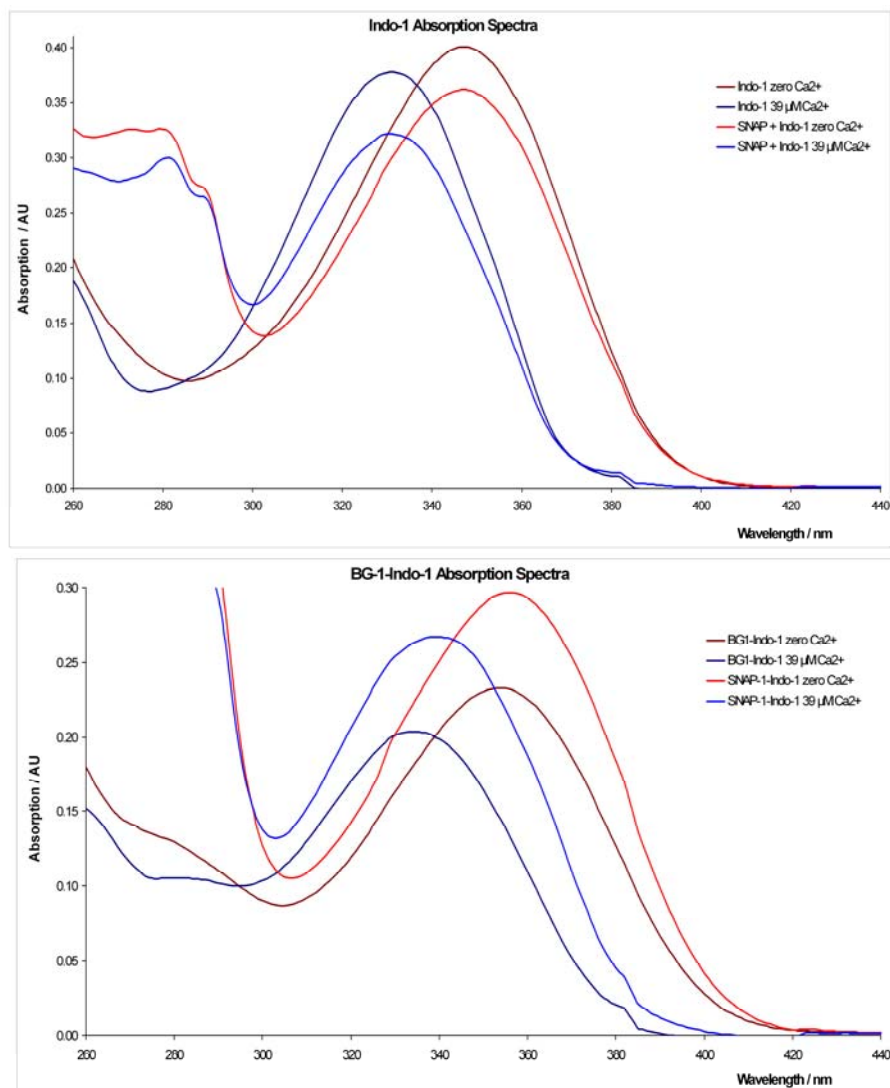
### AM-BG3-Indo-1 (**12**).

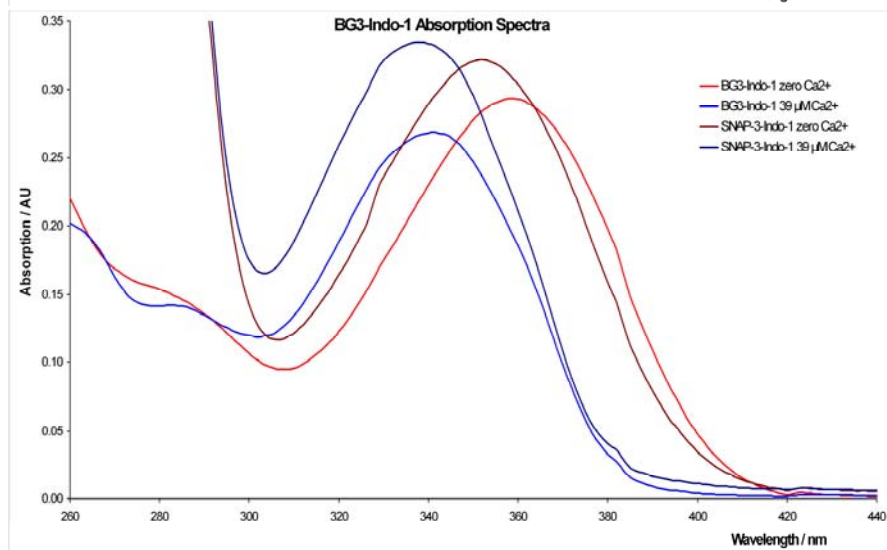
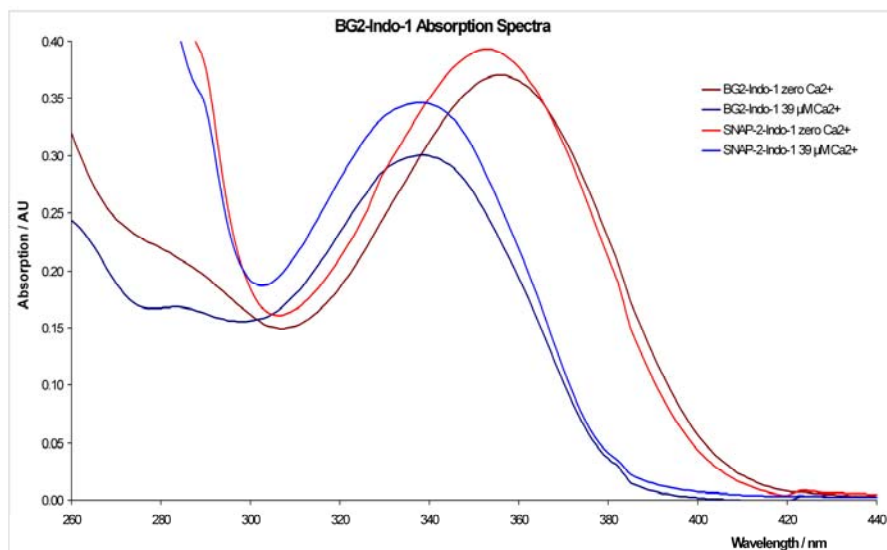


Indo-1 azido AM ester (**11**) (15.5 mg, 15.2  $\mu$ mol) and BG alkyne (**9**) (8.1 mg, 23.1  $\mu$ mol) were solubilized in DCM/*i*PrOH (1:1) (2.0 mL). Water (0.5 mL), an aqueous solution of 1 M  $CuSO_4$  (25  $\mu$ L, 25  $\mu$ mol) and an aqueous solution of 2 M sodium ascorbate (25  $\mu$ L, 50  $\mu$ mol) were added to this solution. The mixture was stirred for 48 h at room temperature. After this period, the suspension was centrifuged to remove the solid particles. The crude product in the supernatant was purified by reversed-phase HPLC on a C18-column using a linear gradient of acetonitrile in water containing 0.1% trifluoroacetic acid and concentrated by lyophilization to yield 15.2 mg (11.1  $\mu$ mol, 73%) of AM-BG3-Indo-1 ester (**12**). The concentration of AM-BG3-Indo-1 (**12**) was estimated to be 2.03 mM after 1 M KOH aqueous hydrolysis to BG3-Indo-1 ester (**10**). The absorption at  $\lambda = 358$  nm was measured as described before.  $^1H$  NMR (400 MHz,  $CD_3OD$ )  $\delta$  7.88 (s, 1H), 7.70 (s, 1H), 7.51 (d,  $J = 8.3$ , 1H), 7.46 – 7.32 (m, 6H), 7.16 (d,  $J = 7.9$ ,

2H), 6.89 (d,  $J = 8.3$ , 1H), 6.82 (s, 1H), 6.79 – 6.73 (m, 2H), 6.68 (d,  $J = 7.6$ , 1H), 5.61 (s, 4H), 5.58 (s, 4H), 5.45 (s, 2H), 4.42 (br, 4H), 4.31 (br, 4H), 4.24 (s, 4H), 4.14 (s, 4H), 3.40 (t,  $J = 6.5$ , 2H), 2.98 (t,  $J = 7.2$ , 2H), 2.54 (t,  $J = 7.2$ , 2H), 2.26 (s, 3H), 2.23 – 2.13 (m, 2H), 2.02 (s, 6H), 1.97 (s, 6H). MS-ESI ( $m/z$ ):  $[M + H]^+$  calcd for  $C_{65}H_{72}N_{13}O_{21}$ , 1370.5; found, 1370.4.

**Figure S1:** Absorption spectra of different Indo-1 derivatives and their conjugates with SNAP-tag; conditions see Materials and Methods.





**Figure S2:** Determination of the  $K_D$  values of the different Indo-1 derivatives by plotting of

$\log \frac{R - R_{\min}}{R_{\max} - R} \cdot \frac{F_{\text{free anion}, 485 \text{ nm}}}{F_{\text{Ca}^{2+} \text{ complex}, 485 \text{ nm}}}$  against  $\log [\text{Ca}^{2+}]_{\text{free}}$  (in nM) according to (5). **A:** Indo-1; **B:** SNAP + Indo-1;

**C:** BG1-Indo-1; **D:** SNAP-1-Indo-1; **E:** BG2-Indo-1; **F:** SNAP-2-Indo-1; **G:** BG3-Indo-1; **H:** SNAP-3-Indo-1.

Plots were obtained using the computer program OriginPro (OriginLab) using the fitting function  $y = a \cdot (x - b)$

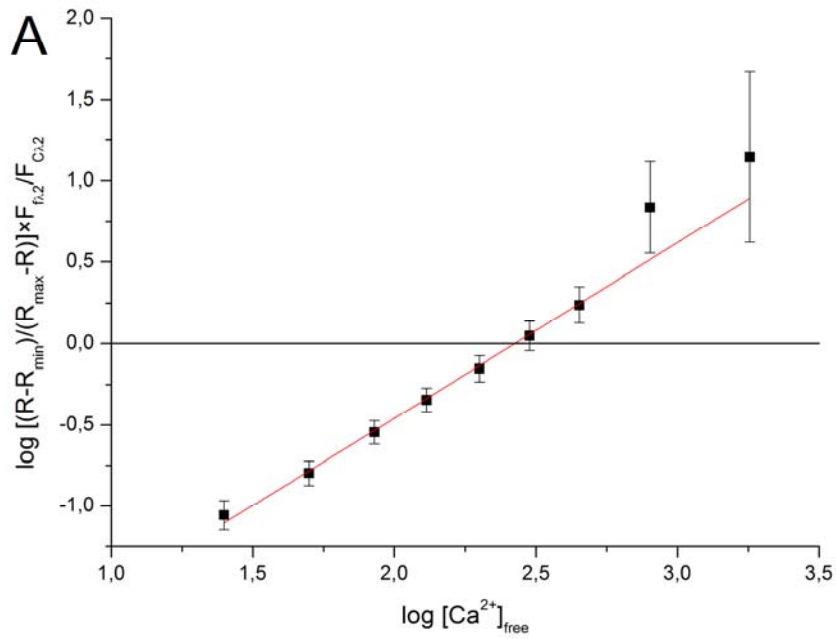
with  $y = \log \frac{R - R_{\min}}{R_{\max} - R} \cdot \frac{F_{\text{free anion}, 485 \text{ nm}}}{F_{\text{Ca}^{2+} \text{ complex}, 485 \text{ nm}}}$ ,  $x = \log [\text{Ca}^{2+}_{\text{free}}]$  and  $K_D^{\text{app}} = 10^b$ . Errors in

$\log \frac{R - R_{\min}}{R_{\max} - R} \cdot \frac{F_{\text{free anion}, 485 \text{ nm}}}{F_{\text{Ca}^{2+} \text{ complex}, 485 \text{ nm}}}$  were estimated by consideration of the error propagation of the uncertainties

in the measured fluorescence values. As weighting constraint in the program the instrumental option was chosen. Errors in  $K_D$  were obtained from the error value obtained for  $b$  by the program. Specifically, the

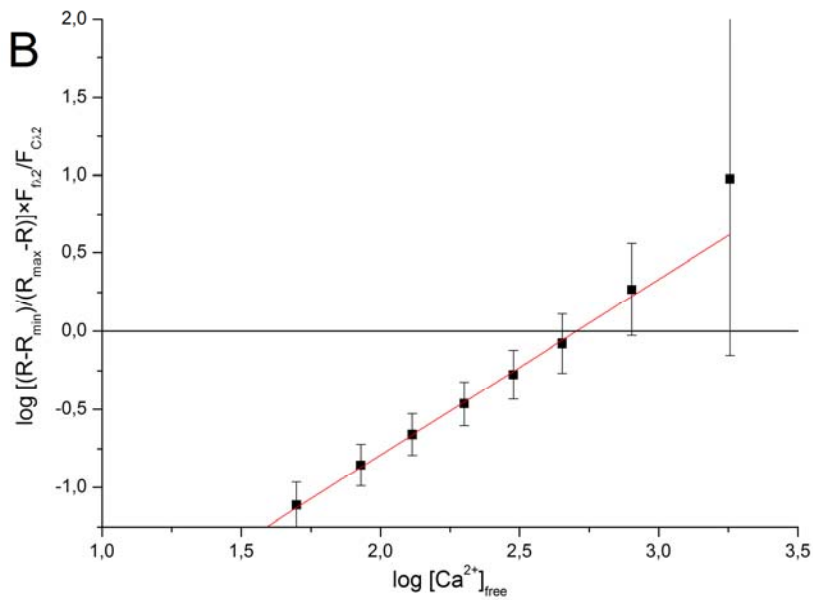
errors given in Table 1 were calculated with the formula  $10^{b+2 \cdot (\text{error of } b)} - 10^b$  using the uncertainty of  $b$  obtained by the fitting program.





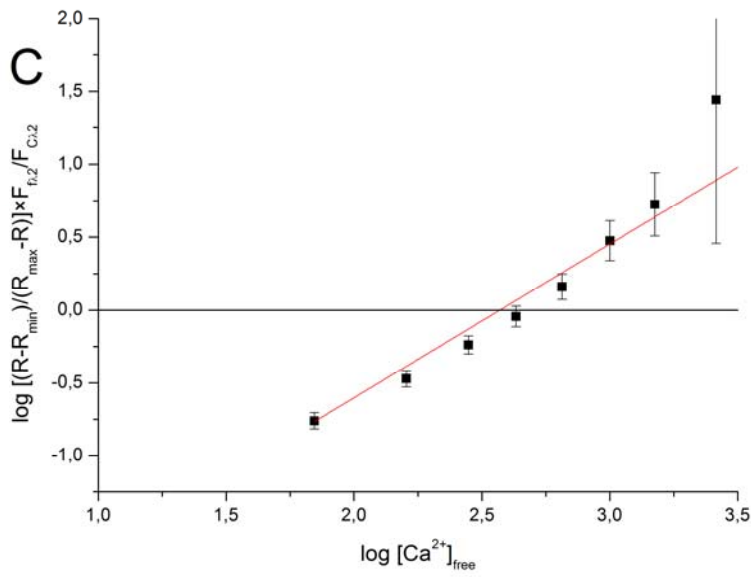
Chi<sup>2</sup>/DoF = 0.28488; R<sup>2</sup> = 0.98894

a	1.07707	0.08066
b	2.42621	0.04001

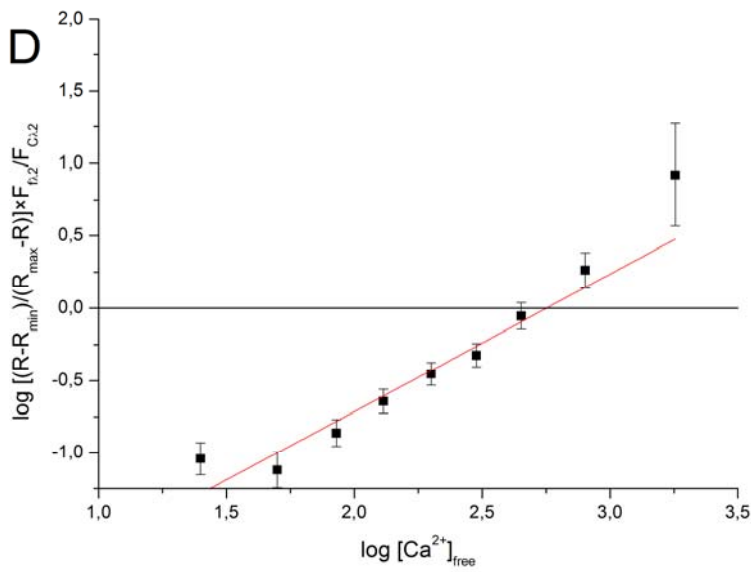


Chi<sup>2</sup>/DoF = 0.02891; R<sup>2</sup> = 0.99642

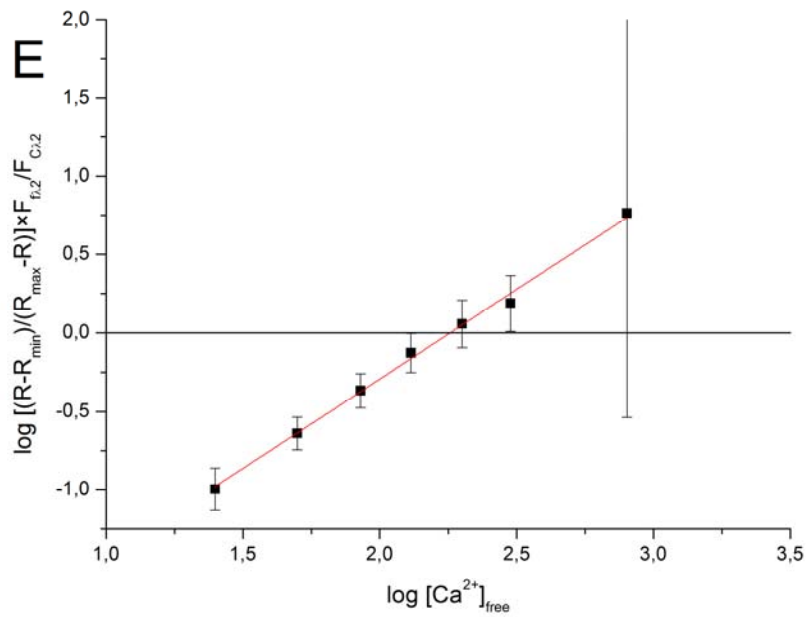
a	1.12286	0.14957
b	2.70484	0.09205



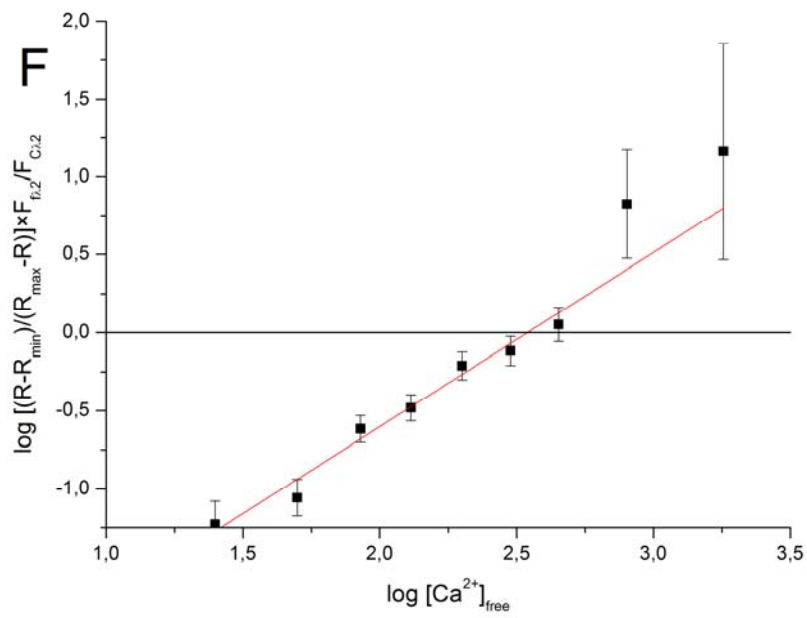
Chi<sup>2</sup>/DoF = 0.06092; R<sup>2</sup> = 0.92997  
a 1.0546 0.10938  
b 2.57082 0.08528



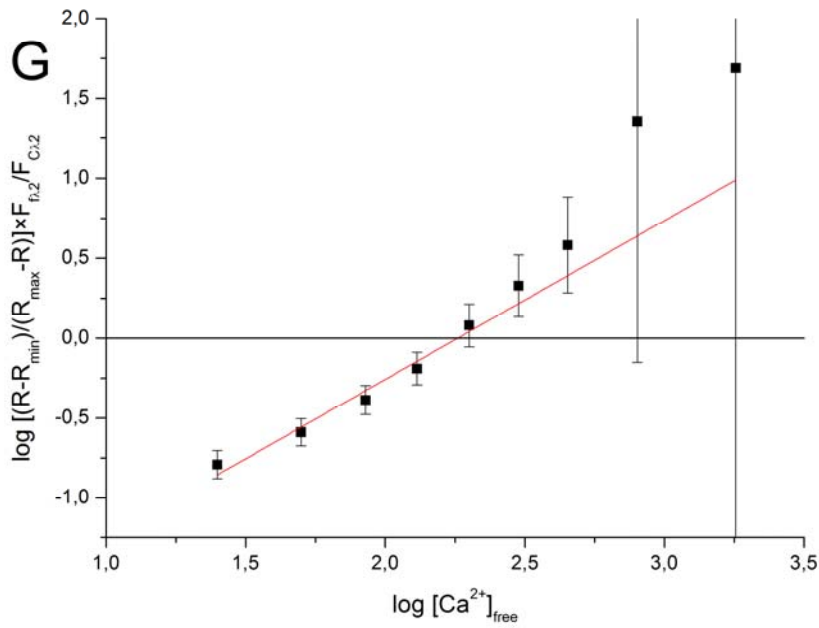
Chi<sup>2</sup>/DoF = 1.43906; R<sup>2</sup> = 0.93394  
a 0.94909 0.07953  
b 2.75288 0.05559



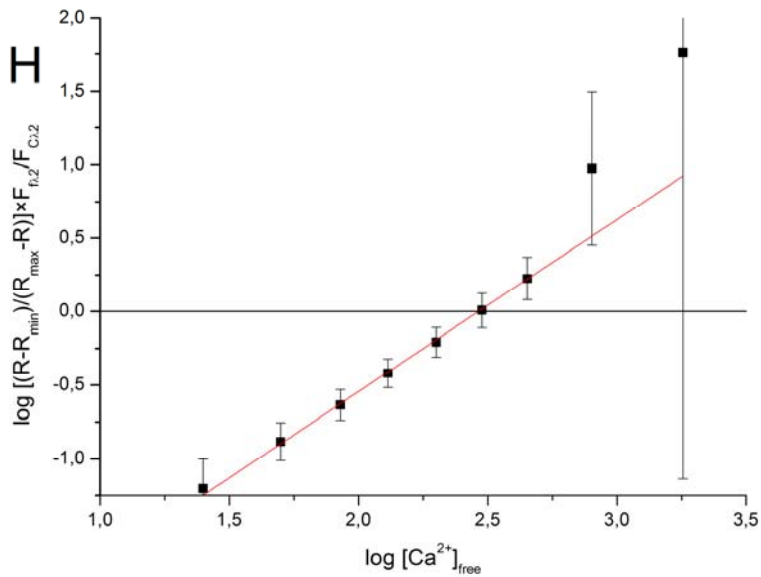
Chi<sup>2</sup>/DoF = 0.04751; R<sup>2</sup> = 0.99529  
 a 1.13959 0.16087  
 b 2.25783 0.06583



Chi<sup>2</sup>/DoF = 0.64435; R<sup>2</sup> = 0.96141  
 a 1.11516 0.1052  
 b 2.54023 0.04912



Chi<sup>2</sup>/DoF = 0.34728; R<sup>2</sup> = 0.96456  
 a 0.99436 0.12225  
 b 2.25996 0.0642



Chi<sup>2</sup>/DoF = 0.13835; R<sup>2</sup> = 0.98737  
 a 1.17133 0.13461  
 b 2.46499 0.05307

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