A pH-correctable, DNA-based fluorescent reporter for organellar Calcium

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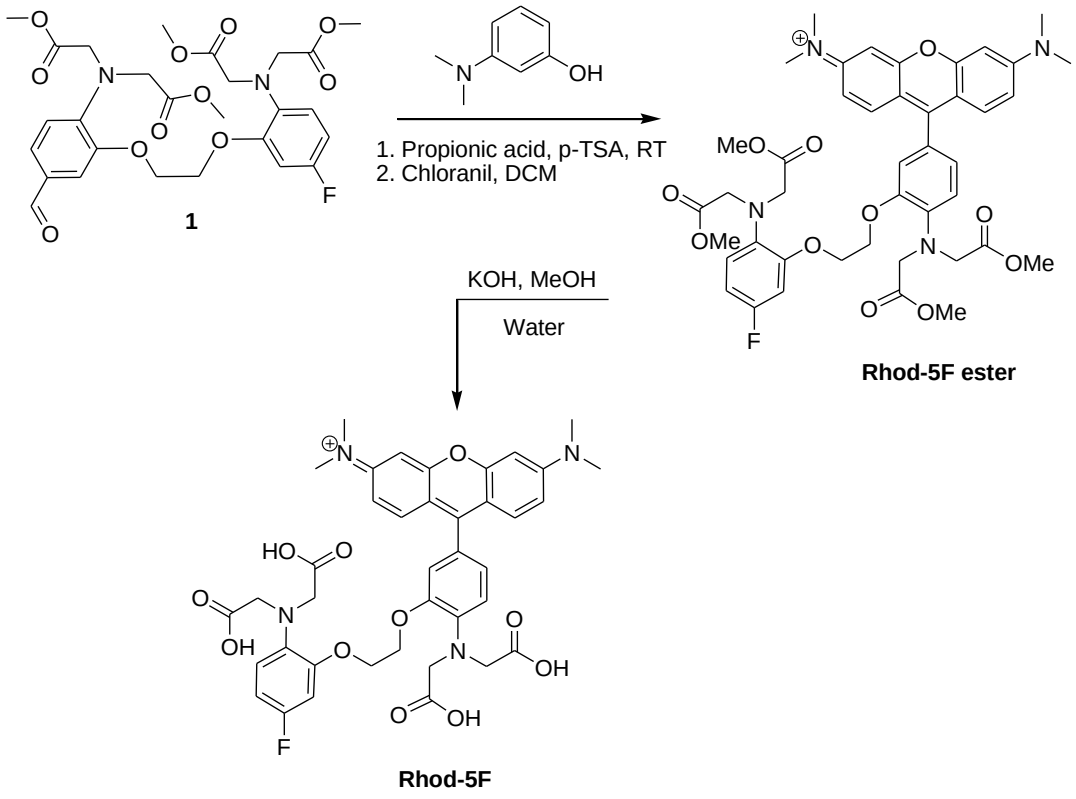
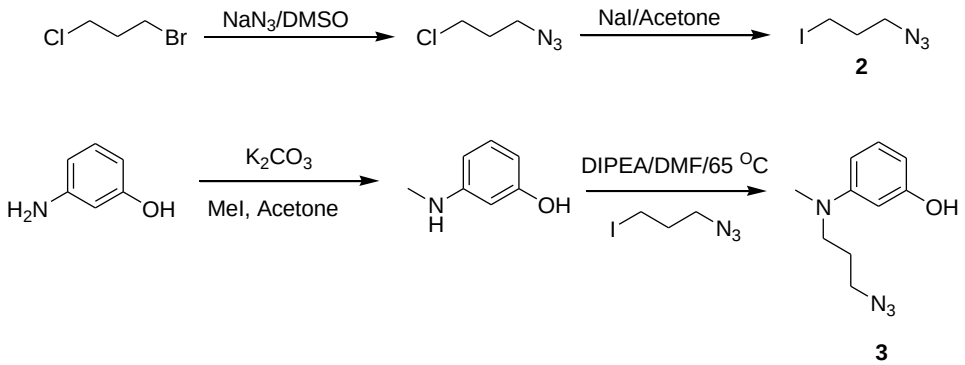
Scheme 1: Synthesis of Rhod-5F



Scheme 2: Synthesis of 3-((3-azidopropyl)(methyl)amino)phenol (**3**).



Scheme 3: Synthesis of Rhod-5F-N_3

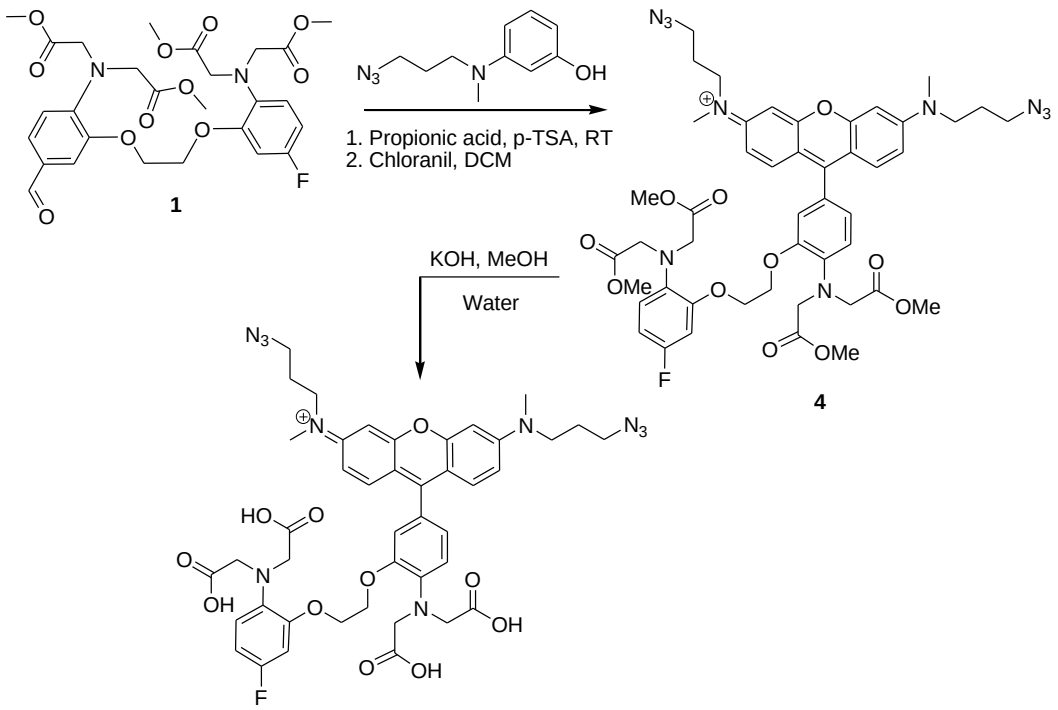


Table S1. Sequences used to form *CalipHluor*, *CalipHluor*$_{Ly}$ and *CalipHluor*^{mLy}. D1 and D2 were used to form *CalipHluor*$_{Ly}$; OG-D1 and D2 were used to form *CalipHluor*^{mLy}. Bromo cytosines in D1 are underlined and highlighted in red. O1-A488, O2-A647 and O3 strands were used to form *CalipHluor*. Complimentary sequences are highlighted in matching colors.

Strand	Sequence information
D1	5'-Alexa 488-CCC CTA ACC CCT AAC CCC TAA CCC CAT ATA TAT CCT AGA ACG ACA GAC AAA CAG TGA GTC-3'
D2	5'-DBCO-GAC TCA CTG TTT GTC TGT CGT TCT AGG ATA /iAlexa 647N/AT ATT TTG TTA TGT GTT ATG TGT TAT-3'
O1-A488	5'-Alexa-488-CCCCAACCCCAATACATTTTACGCCTGGTGCC-3'
O2-A647	5'-CCGACCGCAGGATCCTATAAAACCCCAACCCC-Alexa 647-3'
O3-DBCO	5'-TTA TAG GAT CCT GCG GTC GG/iDBCON/ GGC ACC AGG CGT AAA ATG TA-3'
OG-D1	5'-Oregon Green-AT AAC ACA TAA CAC ATA ACA AAA TAT ATA TCC TAG AAC GAC AGA CAA ACA GTG AGT C-3'

Table S2: Amount of free [Ca^{2+}] in clamping buffer at pH 5.5 was calculated using Maxchelator software.

Added calcium (μM)	Amount calcium added (μL)	Concentration of calcium added	Free [Ca^{2+}] (μM) in 50 μL
0	0	0	0
1	1	50 μM	3.89 E-2
2	2	50 μM	7.80 E-2
10	1	0.5 mM	3.89 E-1
20	2	0.5 mM	7.80 E-1
50	1	2.5 mM	1.9
100	2	2.5 mM	3.9
200	1	10 mM	7.9
500	1	25 mM	20.4
1E3	1	50 mM	43.1
2E3	2	50 mM	96.3
5E3	1	250 mM	360.3
10E3	2	250 mM	1.86 E3
20E3	2	500 mM	10.4 E03

Table S3: Mean pH and free [Ca^{2+}] in EE, LE and Ly of wild type (N2) worms, lysosomes of *catp-6*, *cup-5* +/- and *catp-6* RNAi in *cup-5* +/- worms using *CalipHluor*$_{Ly}$.

Worm	pH	Free [Ca^{2+}] (μM)
EE of N2	6.46 ± 0.07	0.3 ± 0.1
LE of N2	5.95 ± 0.02	0.3 ± 0.1
Ly of N2	5.30 ± 0.02	11 ± 0.8
Ly of *catp-6*	5.47 ± 0.03	1.6 ± 0.4
Ly of *cup-5* +/-	5.15 ± 0.01	40 ± 1.5
Ly of CATP-6 RNAi in *cup-5* +/-	5.50 ± 0.10	16 ± 4.9

Early endosome (EE), Late endosome (LE) and Lysosomes (Ly)

For all experiments n = 15 cells, 50 endosomes; data represent the mean ± s.e.m. Experiments were repeated thrice independently with similar results.