Contribution to *Analyst* Special Issue

Supporting Information

A Membrane-Activatable Near-Infrared Fluorescent Probe with Ultra-Photostability for Mitochondrial Membrane Potential

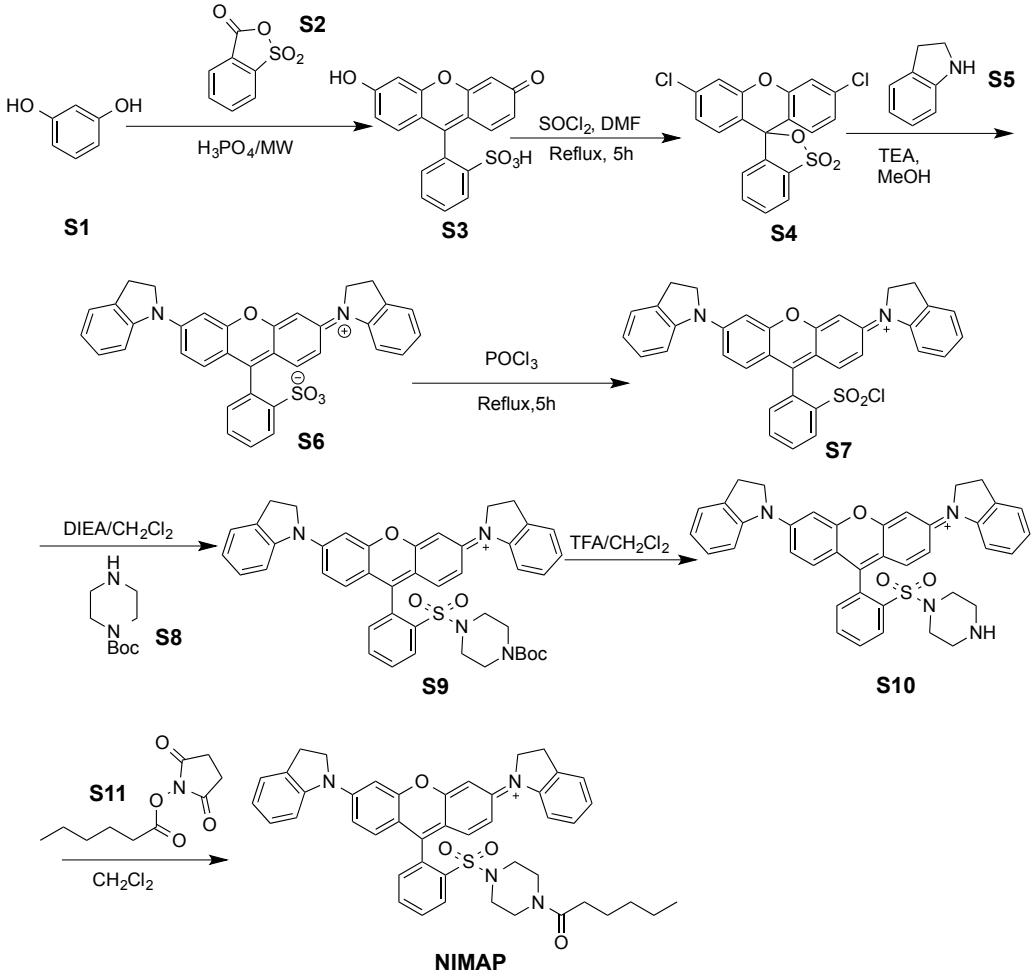
Wei Ren,[a‡] Ao Ji,[a‡] Omran Karmach,[b] David G. Carter,[c] Manuela M. Martins-Green,[b]* and Hui-wang Ai[a]*

[a] Department of Chemistry, [b] Department of Cell Biology & Neuroscience, and [c] Institute for Genome Biology, University of California Riverside, 900 University Ave, Riverside, CA 92521

[‡]These two authors contributed equally.

*Corresponding Author E-mails: huiwang.ai@ucr.edu, and manuela.martins@ucr.edu

Synthesis of NIMAP



Scheme S1 Synthetic route to prepare NIMAP

Compounds **S1**, **S2**, and **S8** are commercially available. They were purchased from Sigma-Aldrich or Acros Organics without further purification. Sulfonefluorescein (**S3**) was prepared from resorcinol (**S1**) and 2-sulfobenzoic cyclic anhydride (**S2**) under microwave irradiation following a published procedure [1], and used directly. Compounds **S4**, **S6** and **S7** were also prepared according to the literature [2]. Next, DIEA (18 μL, 0.1 mmol) and **S8** (19 mg, 0.1 mmol) were added into freshly prepared compound **S7** (14.2 mg, 0.025 mmol) in CH_2Cl_2 (10 mL). The reaction was stirred at RT for 5 h and monitored with TLC. The mixture was washed twice with diluted HCl (1 M, 5 mL), and once with H_2O (10 mL). The organic layer was dried over Na_2SO_4 and concentrated in vacuo to yield crude **S9** as dark blue solid. Without further purification, **S9** was dissolved in TFA/CH_2Cl_2 (1:1). The mixture was stirred at RT for 30 min before the solvent was removed. The derived crude **S10** was re-dissolved in CH_2Cl_2 (10 mL), to which TEA (14 μL, 0.1 mmol) and **S11** (22 mg, 0.1 mmol) in CH_2Cl_2 (2 mL) was introduced. The resulting solution was stirred for another 10 h at RT. The reaction mixture was washed with diluted HCl (1 M, 5 mL) twice and H_2O (10 mL) once. The organic layer was dried over Na_2SO_4 and concentrated in vacuo. The crude NIMAP was purified by column chromatography (CH_2Cl_2/MeOH = 100:3) to yield dark blue solid (4.6 mg, 0.033 mmol). The total yield was 18% over all steps.

NIMAP: 1H NMR (400 MHz, $CDCl_3$): δ 8.1 (d, J1 = 7.4 Hz, 1H), 7.91-7.82 (m, 2H), 7.59-7.46 (m, 7H), 7.33-7.24 (m, 6H), 7.11 (t, *J* = 8 Hz, 2H), 4.36 (m , 4H), 3.47 (m, 4H), 3.3 (t, J = 7.6 Hz, 4H), 3.01 (m, 2H), 2.99 (m, 2H), 2.27 (t, J = 8.4 Hz, 2H), 1.5 (m, 2H), 1.25 (m, 4H), 0.81 (t, J = 7.2 Hz, 3H). 13C NMR (100 MHz, $CDCl_3$): δ 172.5, 157.8, 156, 152.7, 142.4, 138.6, 135, 133.9, 132.2, 131.8, 131.4, 130.9, 129.8, 127.9, 126.5, 125.3, 118.2, 117, 113.9, 100.5, 53.6, 45.9, 45.6, 45.3, 41.3, 33.3, 31.7, 29.9, 28.4, 25.7, 24.9, 22.6, 14.1.

ESI-MS $[M]^+$ calcd: 737.46, found: 737.44

References

[1] Cihelnik, S.; Stibor, I.; Lhotak, P. *Collect. Czech. Chem. Commun*, 2002, 67, 1779-1789.

[2] Takahashi, S.; Piao, W.; Matsumura Yuriko.; Komatsu, Toru.; Ueno, T.; Terai, T.; Kamachi, T.; Kohno, M.; Nagano, T.; Hanaoka, K. *J. Am. Chem. Soc.* 2012, 134, 19588-19591.

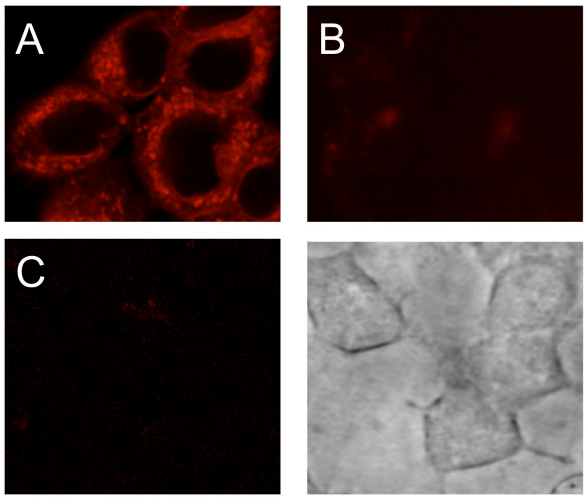


Figure S1. Fluorescence images of HEK 293T cells stained with (**A**) 100 μM **2** or (**B**) 100 μM **3** at 37°C for 45 min. For comparison, (**C**) fluorescence and (**D**) bright-filed images of unstained HEK 293T cells are also shown.

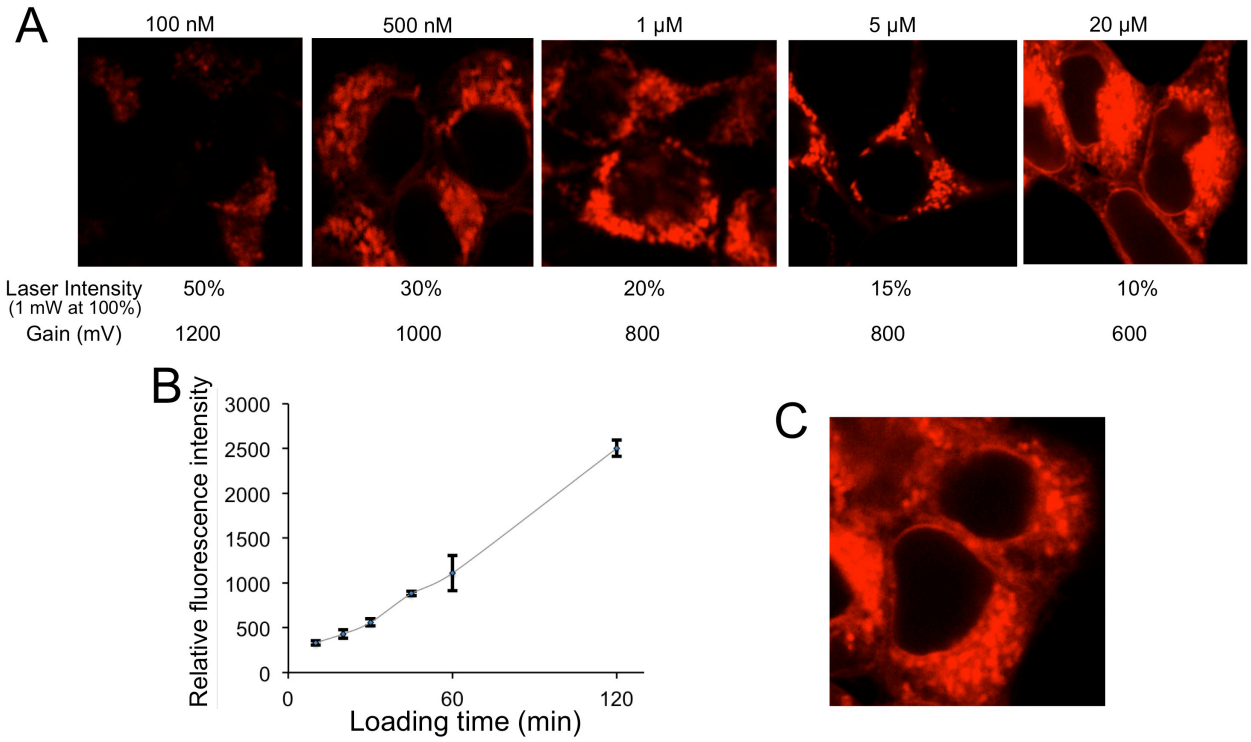


Figure S2. (A) Fluorescence images of HEK 293T cells stained with NIMAP at the indicated concentrations at 37°C for 45 min. Laser intensities and gain settings are also shown on the bottom of each image. (B) Fluorescence intensities of mitochondria in HEK 293T cells stained with 1 μM NIMAP for the indicated incubation time. (C) A fluorescence image of HEK 293T cells stained with 1 μM NIMAP for 2 h.

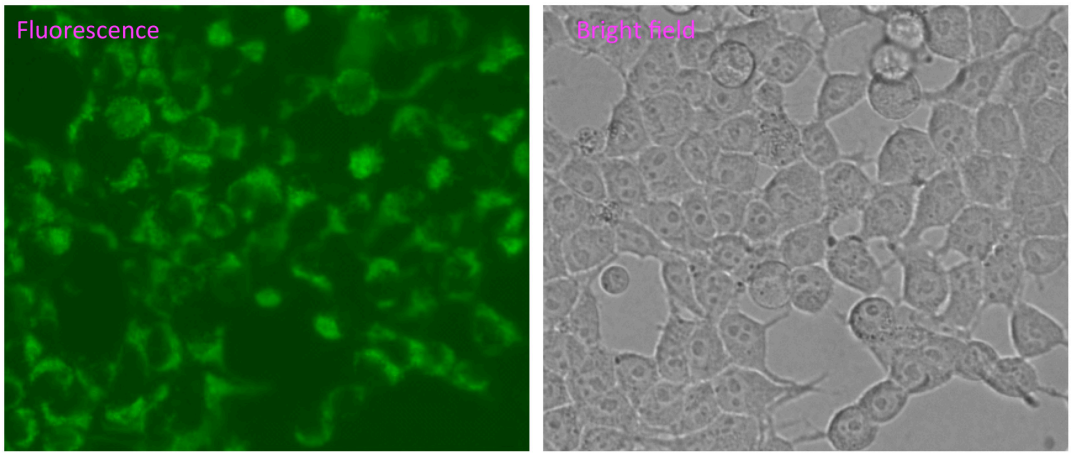


Figure S3. Fluorescence images of HEK 293T cells stained with 5 μM Rhodamine 123 at 37°C for 1 h, and exchanged into fresh 1x DPBS without additional wash steps. Typical Rhodamine 123-staining procedures require multiple rounds of wash steps to gain good contrast. The images were taken on a Motic AE31 inverted microscope, equipped with a 480/40 excitation filter, a 535/50 emission filter, and a 505 LP dichroic filter (Chroma).

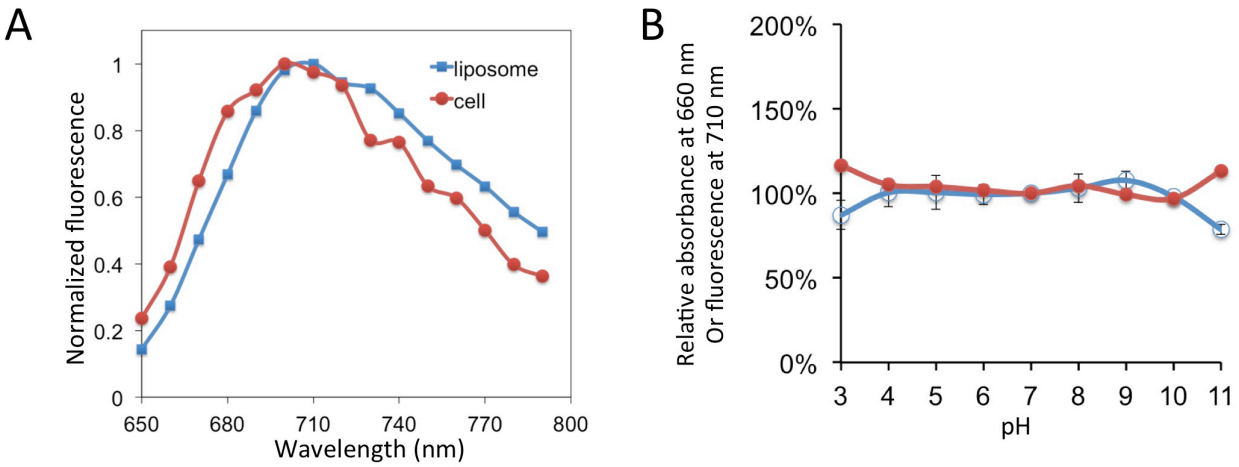


Figure S4. (A) Normalized fluorescence of NIMAP in packed liposomes (100 μM) or loaded to mitochondria in living HEK 293T cells (5 μM), showing similar emission profiles. The former was recorded on a BioTek Synergy Mx Microplate Reader. The latter spectrum was extracted from spectral imaging data on a Leica SP5. (B) Absorbance of NIMAP (red) at 660 nm in aqueous buffers at various pH values, normalized to the absorbance at pH 7. Also shown is the fluorescence of NIMAP (blue) at 710 nm (excitation at 650 nm) in aqueous buffers supplemented with 90% glycerol (v/v), normalized to the value at pH 7.

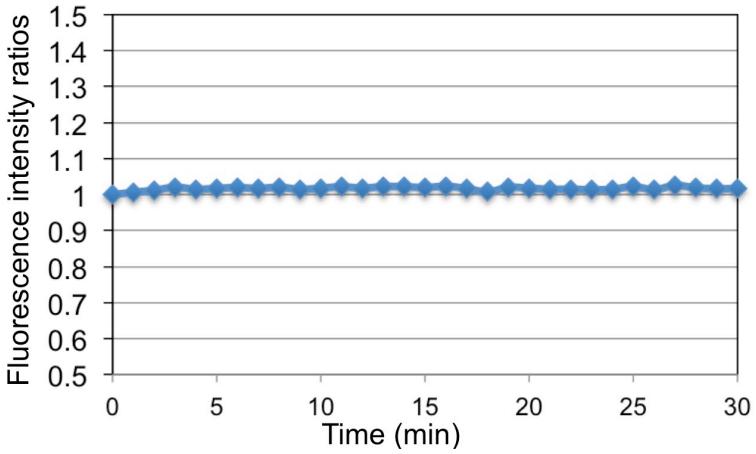


Figure S5. Time-lapse fluorescence of NIMAP (100 μM) in packed liposomes in response to 0.2‰ H_2O_2. The data are represented as fluorescence ratios of the groups treated or untreated with H_2O_2 at given time points.

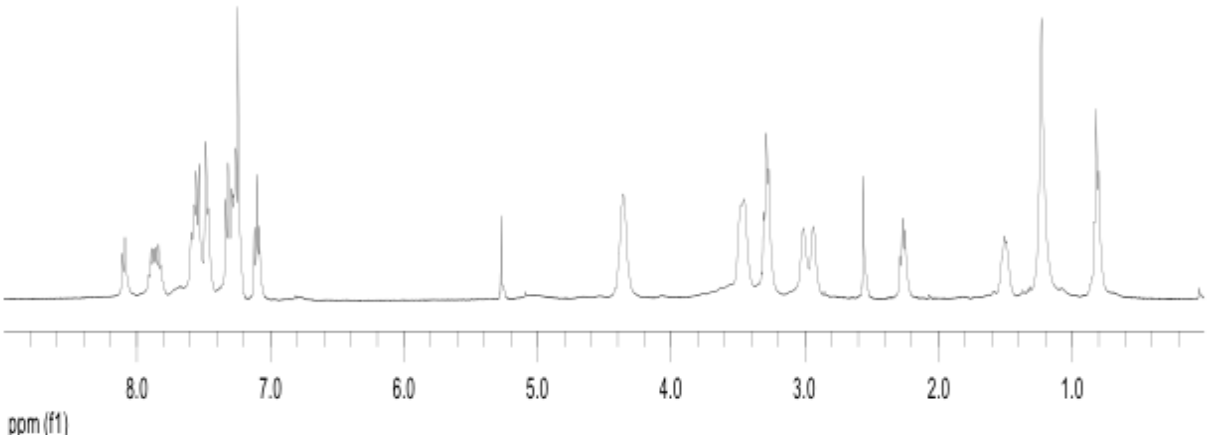
MOVIE S1

Time-lapse fluorescence imaging of NIMAP-loaded HeLa cells, stimulated with oligomycin and FCCP. The movie was rendered at two frames per second, with each frame representing one-minute intervals of a 40-minute recording.

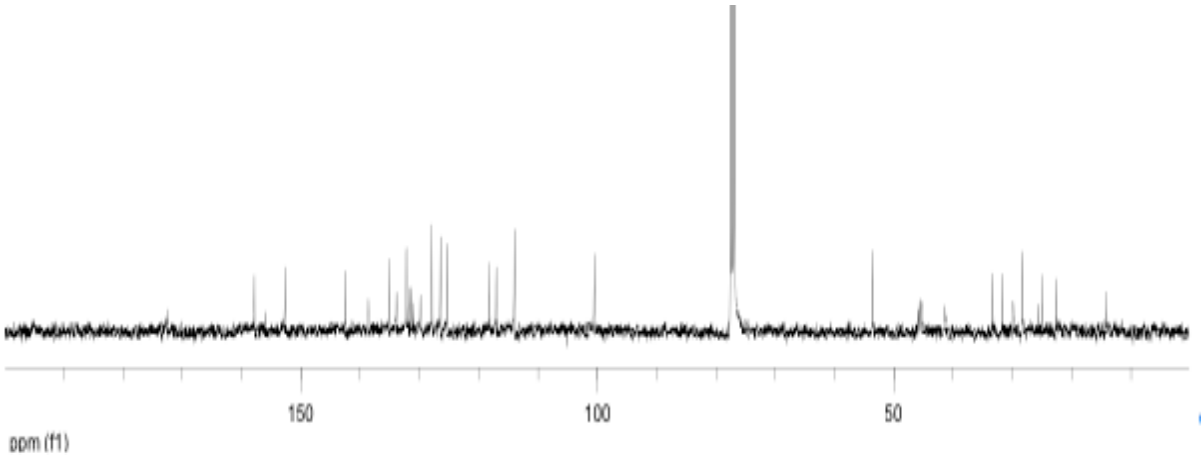
MOVIE S2

Time-lapse fluorescence imaging of NIMAP-loaded HEK 293T cells, stimulated with H_2O_2. The movie was rendered at two frames per second, with each frame representing one-minute intervals of a 15-minute recording.

1H NMR for NIMAP



13C NMR for NIMAP



MS for for NIMAP