Literature Report

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Communication

Imaging Reversible Mitochondrial Membrane Potential Dynamics with a Masked Rhodamine Voltage Reporter

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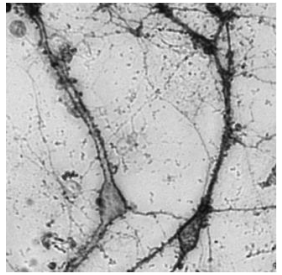
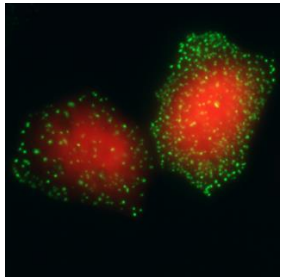
Voltage Imaging



Activity Tracing



B.S. Biology/Chemistry, B.A. Philosophy/Theology,
Point Loma Nazarene University, 2004

Ph.D. Chemistry,
University of California, Berkeley, 2009 (Christopher J. Chang, advisor)

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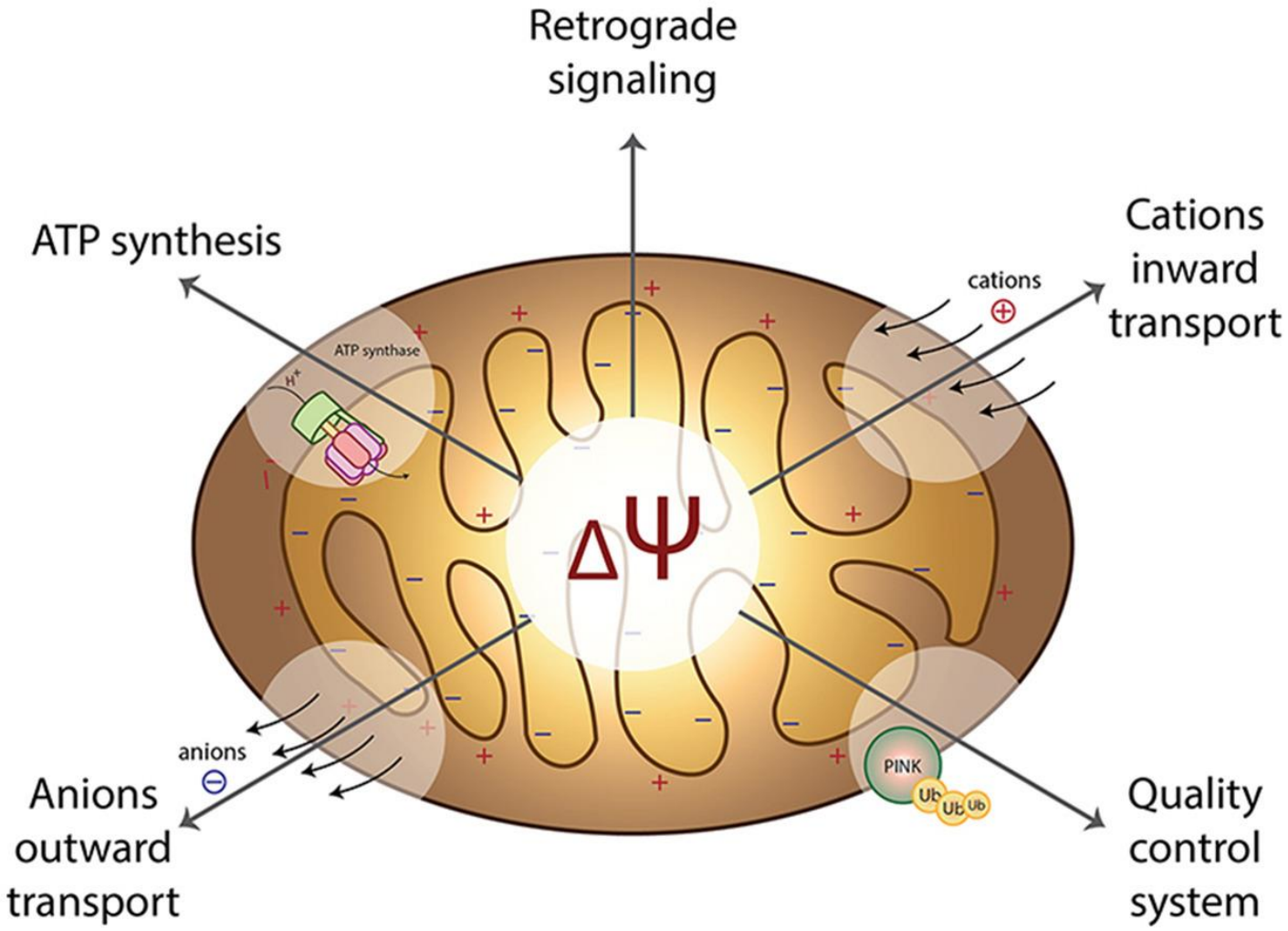
Assistant Professor,
Chemistry and Molecular & Cell Biology, University of California, Berkeley, 2013

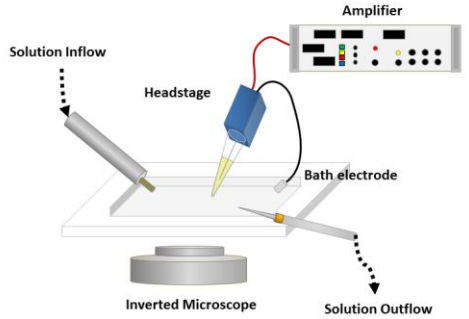
Associate Professor,
Chemistry and Molecular & Cell Biology, University of California, Berkeley, 2020

Mitochondrial membrane potential

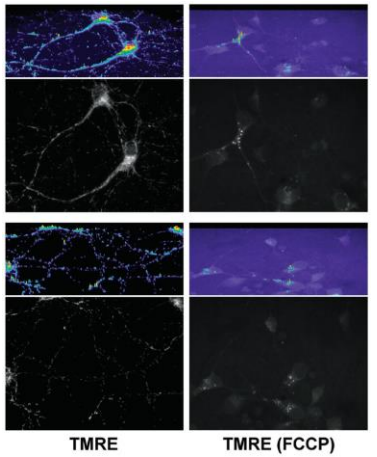


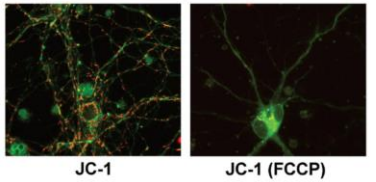
线粒体膜电位（ΔΨm）是由与Krebs循环的活性相关的氧化还原转换产生的，并且是能量合成的中间形式，被ATP合成酶用来制造ATP。这些转变不仅产生电势，而且还产生质子梯度，并且它们共同形成氢离子的跨膜电势。

Patch-clamp Electrophysiology



a lipophilic, cationic fluorophore
(in proportion to the negative $\Delta\Psi_m$)





Technical limitations:

Self-quench

Bath solution

Diffusion

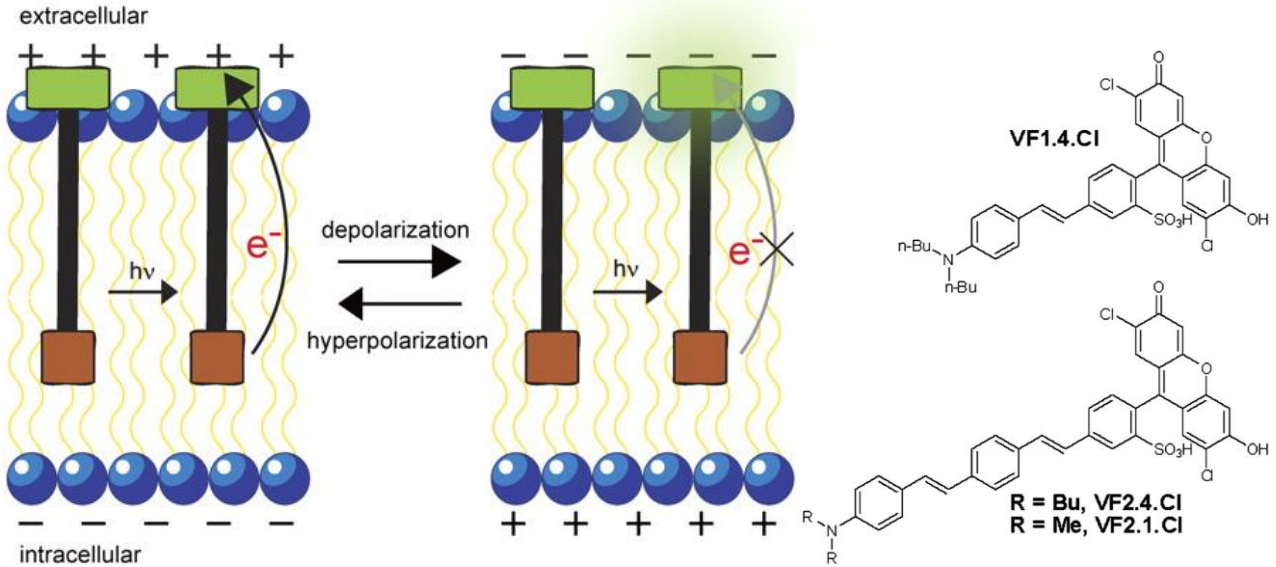
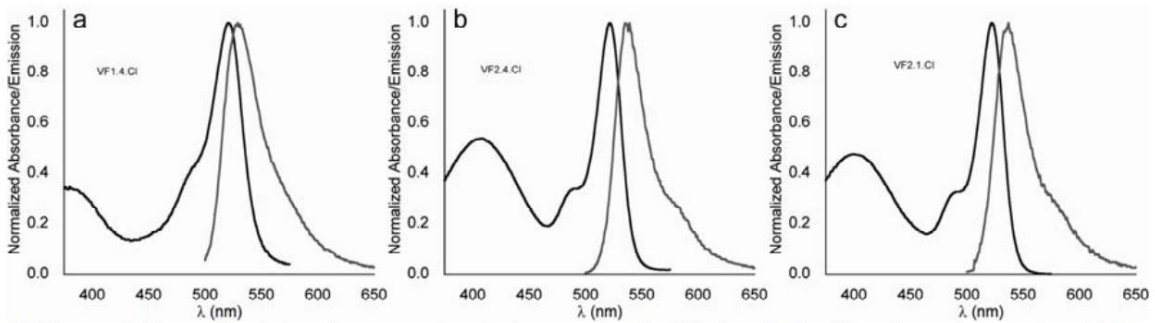


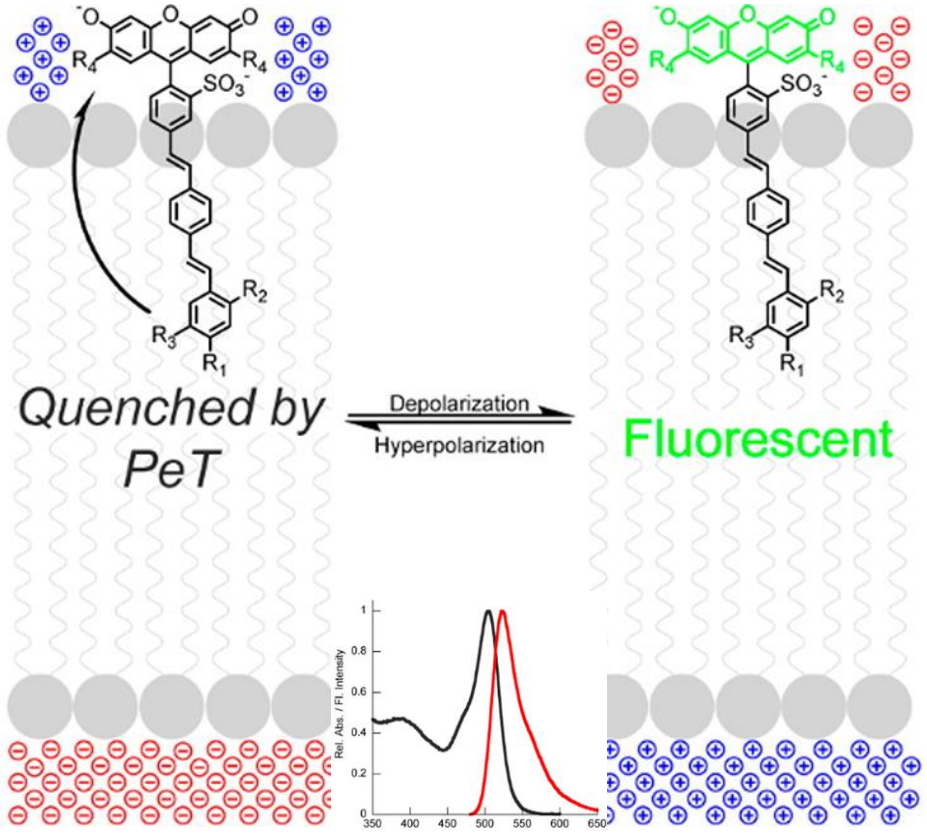
Photo-induced Electron Transfer



SI Figure 1: Normalized absorbance and emission spectra for VF dyes in 5 mM sodium phosphate, pH 9 with 0.1% Triton X-100. Black lines are absorbance spectra; grey lines are emission spectra. a) VF1.4.Cl, b) VF2.4.Cl, and c) VF2.1.Cl.

VF1.4.Cl: λ_{max} = 521 nm, ε = 93,000 $M^{-1}\cdot cm^{-1}$, λ_{em} = 534 nm, Φ = 0.24
VF2.4.Cl: λ_{max} = 522 nm, ε = 97,000 $M^{-1}\cdot cm^{-1}$, λ_{em} = 536 nm, Φ = 0.054
VF2.1.Cl: λ_{max} = 522 nm, ε = 98 000 $M^{-1}\cdot cm^{-1}$, λ_{em} = 535 nm, Φ = 0.057

PNAS, 2012, 2114-2119.



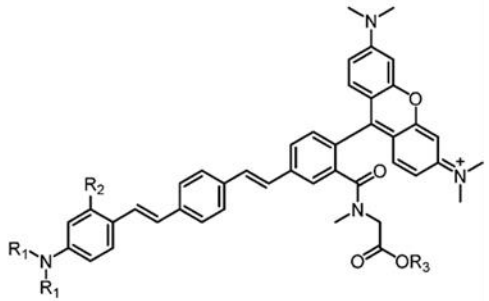
compound	R_1	R_2	R_3	R_4	E (D^+/D) (V)[a]	E (A/A^-) (V)[a]	λ_{abs} (nm)[b]	λ_{em} (nm)[b]	ΔG_{00} (eV)	$\Delta G_{PeT} + w$ (eV)	% $\Delta F/F$ per 100 mV[c]	Φ_{Fl}[b]
VF2.1(diOMe).Cl	$N(Me)_2$	OMe	OMe	Cl	0.033	−2.02	521	535	2.38	−0.325	20	0.26
VF2.1(OMe).Cl	$N(Me)_2$	OMe	H	Cl	0.090	−2.02	522	536	2.38	−0.263	49	0.13
VF2.1(OMe).F	$N(Me)_2$	OMe	H	F	0.090	−2.11	509	528	2.44	−0.243	44	0.05
VF2.1.Cl	$N(Me)_2$	H	H	Cl	0.129	−2.02	522	536	2.38	−0.224	27	0.05
VF2.1.F	$N(Me)_2$	H	H	F	0.129	−2.11	508	524	2.44	−0.209	30	0.10
VF2.1(diOMe).H	$N(Me)_2$	OMe	OMe	H	0.033	−2.24	504	522	2.46	−0.186	30	0.24
VF2.1(OMe).H	$N(Me)_2$	OMe	H	H	**0.090**	**−2.24**	**504**	**524**	**2.46**	**−0.130**	**48**	**0.04**
VF2.1.H	$N(Me)_2$	H	H	H	0.129	−2.24	507	528	2.45	−0.076	16	0.11
VF2.1(OMe).Me	$N(Me)_2$	OMe	H	Me	0.090	−2.32	515	536	2.41	0.003	13	0.04
VF2.1.Me	$N(Me)_2$	H	H	Me	0.129	−2.32	513	532	2.42	0.033	5	0.38
VF2.0.Cl	H	H	H	Cl	1.080[d]	−2.02	521	538	2.38	0.722	0	0.50

[a]vs Ferrocene (Fc). [b]0.01% Triton X-100, 5 mM sodium phosphate, pH 9. [c]Measured in voltage-clamped HEK cells. [d]Oxidation potential of stilbene taken from ref 33.

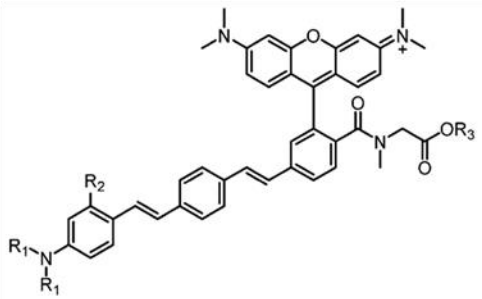
J. Am. Chem. Soc. 2015, 1817–1824

Rhodamine **V**oltage **R**eporters (RhoVRs)

prohibitive dye internalization → *improved membrane localization* → *voltage imaging in hippocampal neurons*



16 R_1=Me R_2=H R_3=H
17 R_1=Et R_2=OMe R_3=H

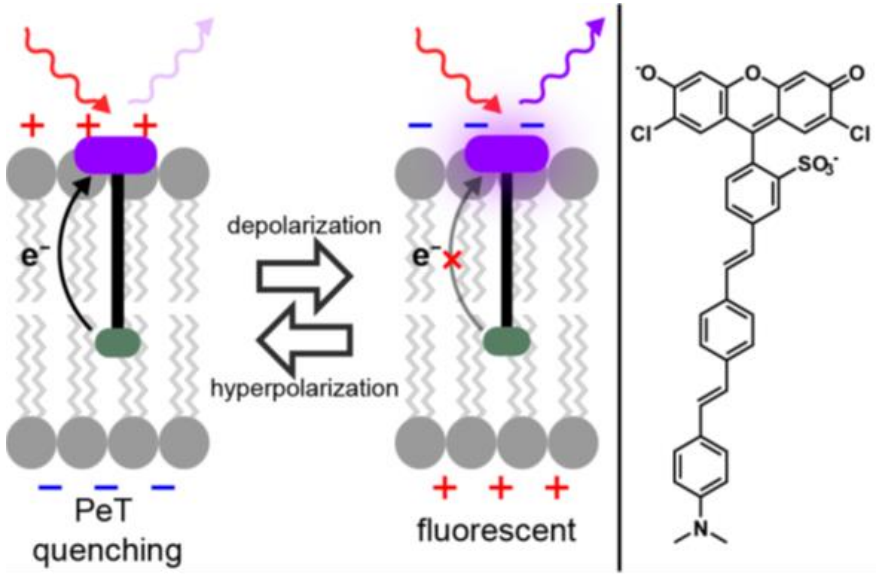


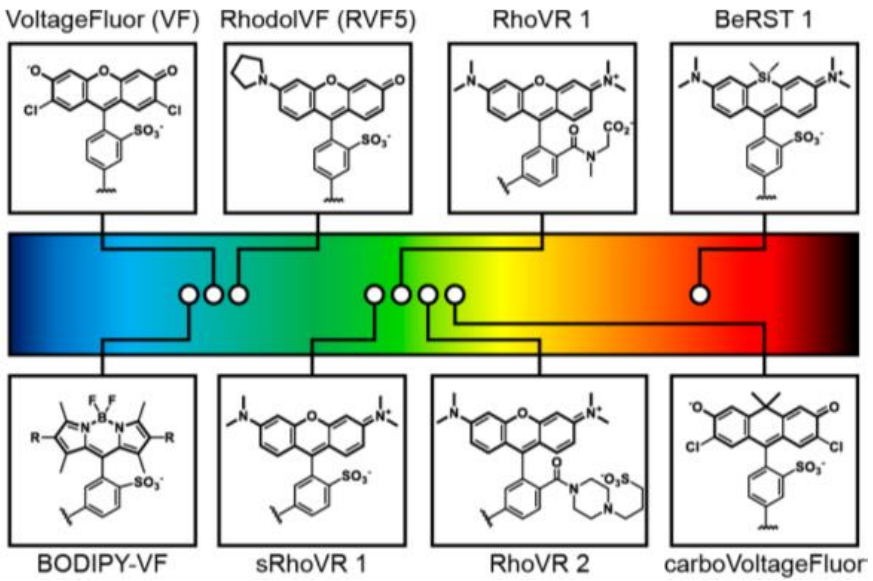
20 R_1=Me R_2=H R_3=H
21 R_1=Et R_2=OMe R_3=H (**RhOVR 1**)

compd	ε/M^{-1} cm^{-1} (λ_{max}/nm)[a]	Φ (λ_{max}/nm)[a]	$\Delta F/F$ (100 mV)[b]	SNR (100 mV)[b]
16	75000 (565)	0.036 (586)	3 ± 0.2%	19:1
17	70000 (565)	0.092 (588)	26 ± 3%	37:1
20	77000 (564)	0.0089 (586)	7 ± 1%	96:1
21	87000 (564)	0.045 (588)	47 ± 3%	160:1

[a]PBS, pH 7.2, 0.1% SDS. [b]Voltage-clamped HEK cells.

J. Am. Chem. Soc. 2016, 9085−9088

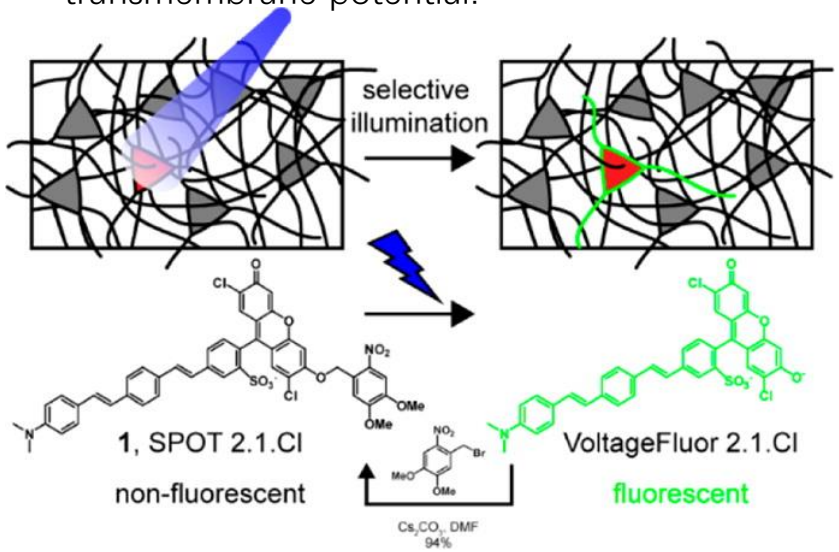




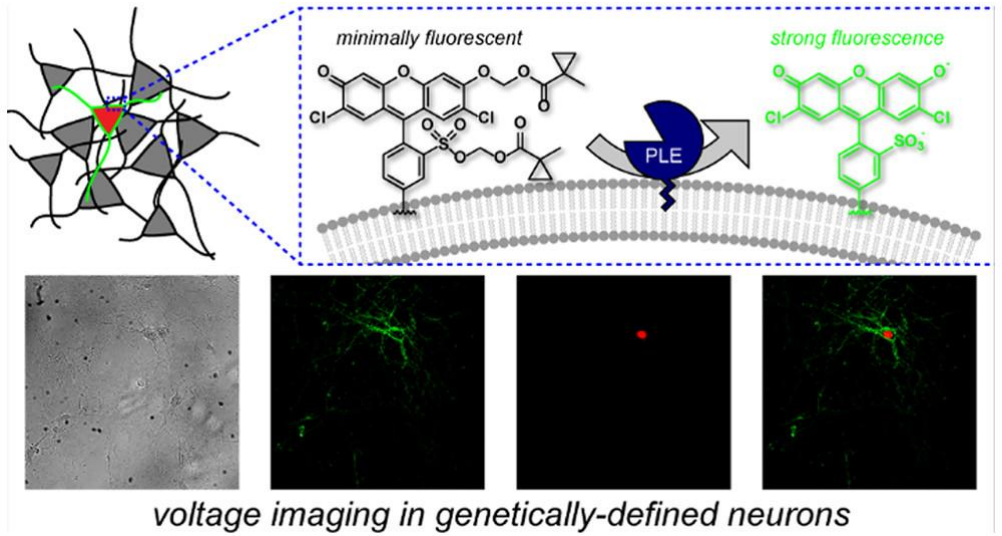
Acc. Chem. Res. 2020, 11−19

Targeting, Including Fluorogenic and Covalent Strategies

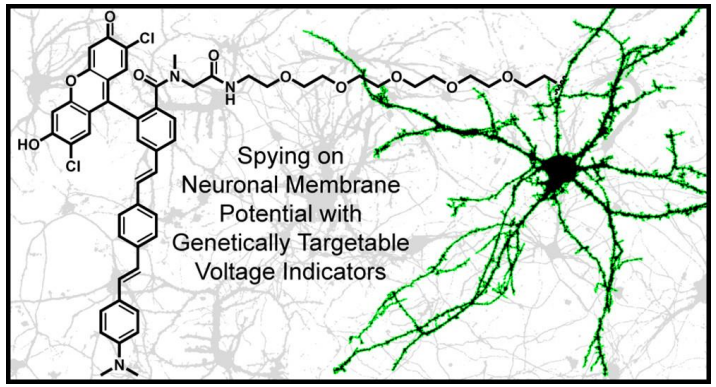
small-molecule photoactivatable optical sensors of transmembrane potential.



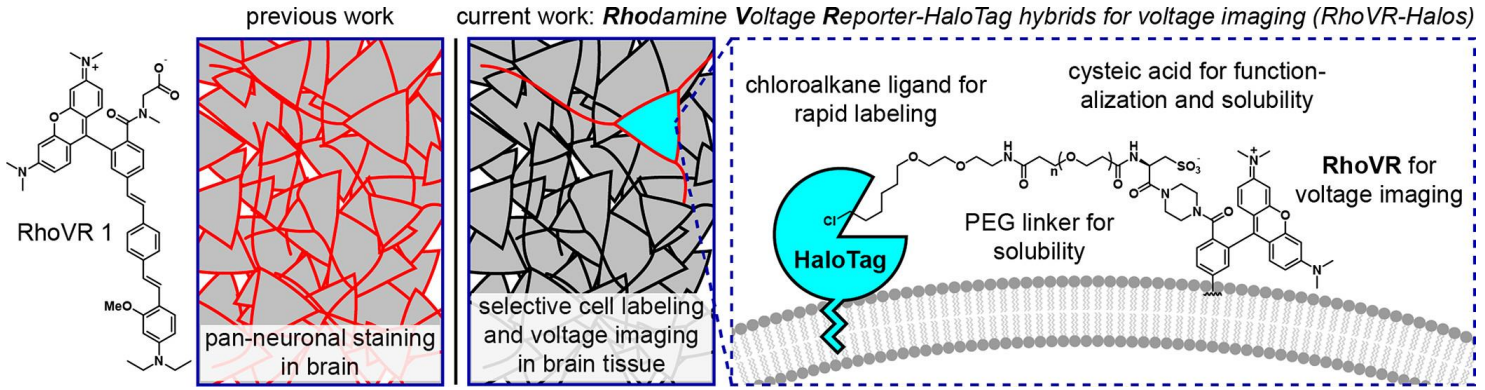
J. Am. Chem. Soc. 2015, 10894– 10897



J. Am. Chem. Soc. 2017, 17334– 17340

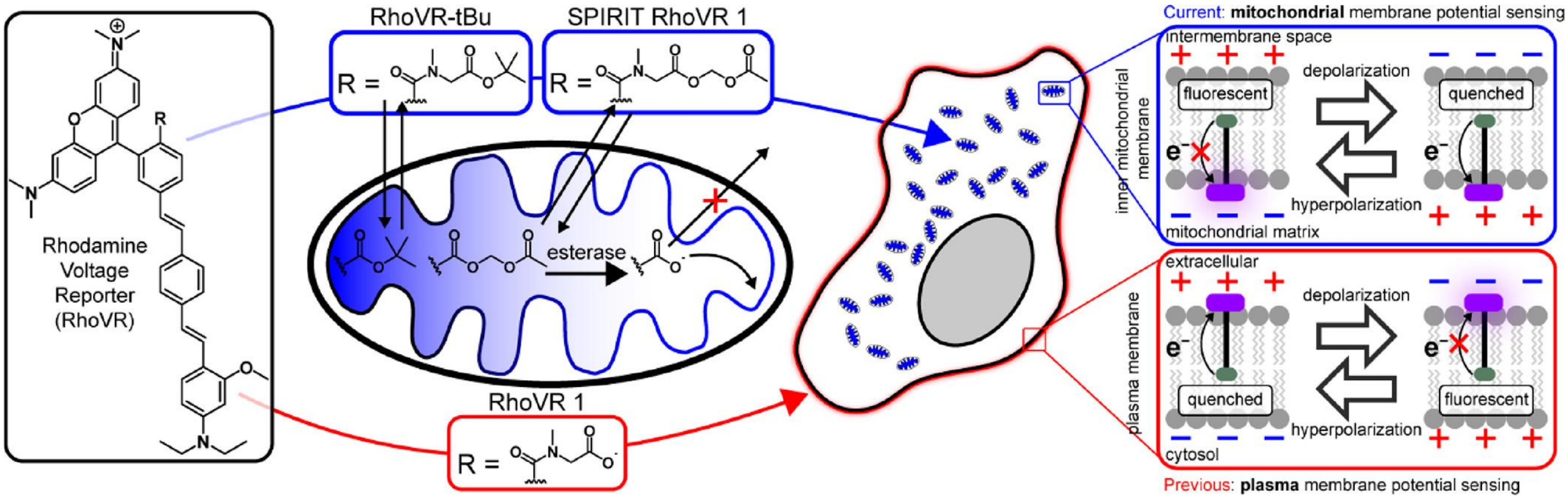


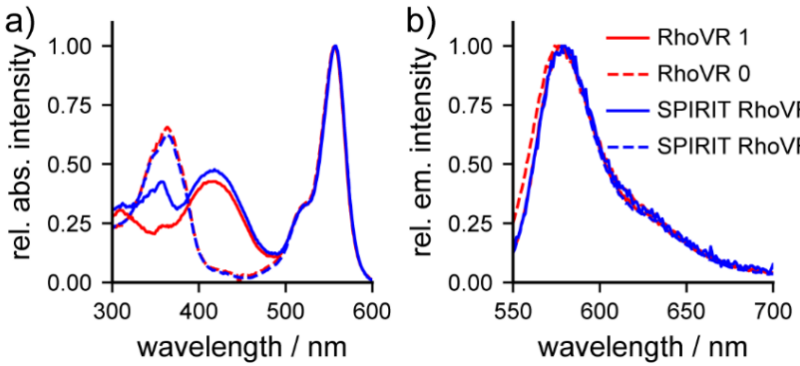
J. Am. Chem. Soc. 2019, 1349– 1358

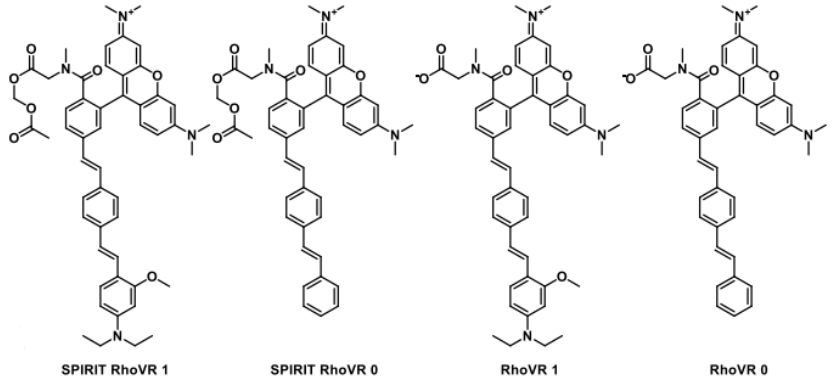


J. Am. Chem. Soc. 2020, 614–622

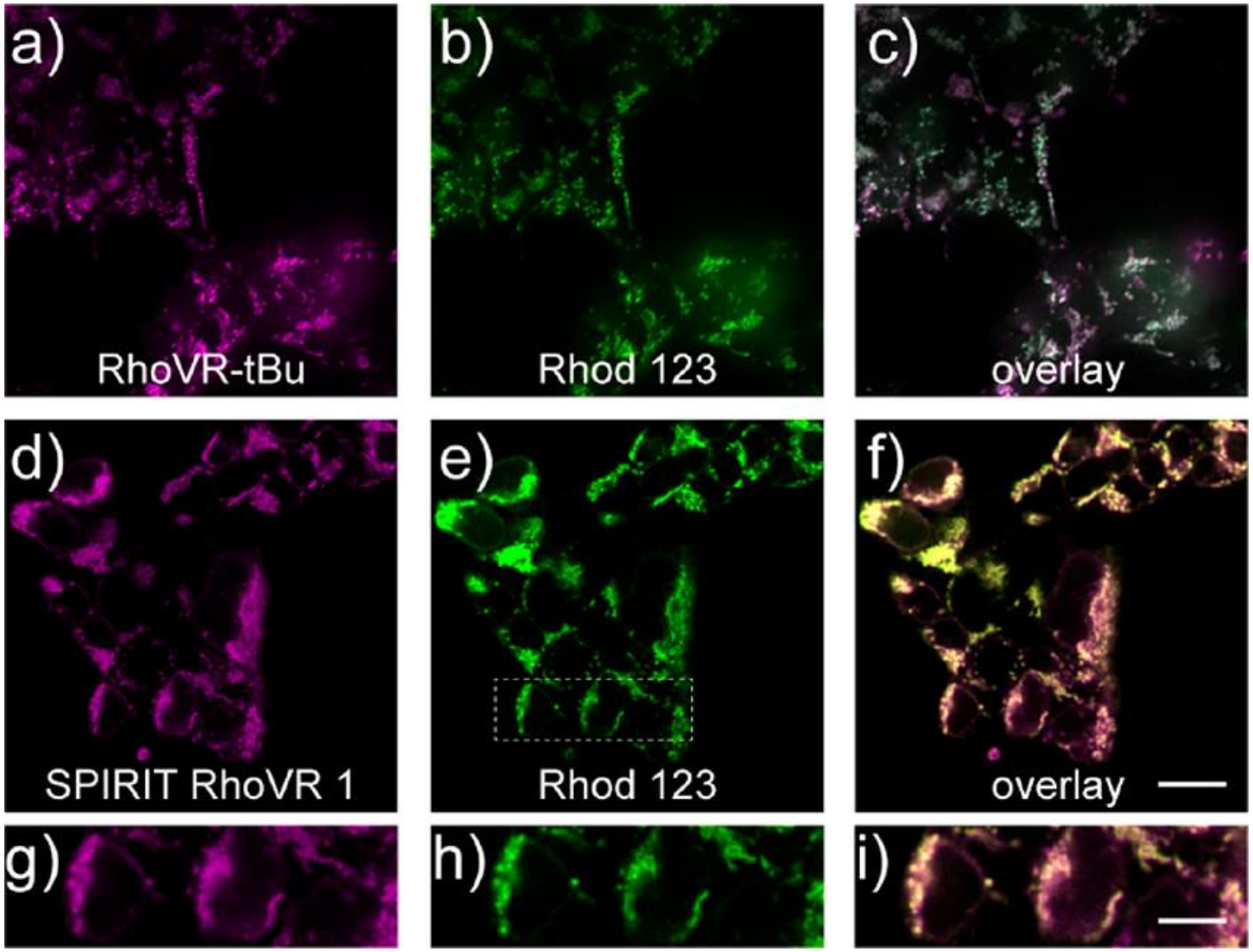
Imaging Mitochondrial Membrane Potential Dynamics with Permeable SPIRIT RhoVR Indicators

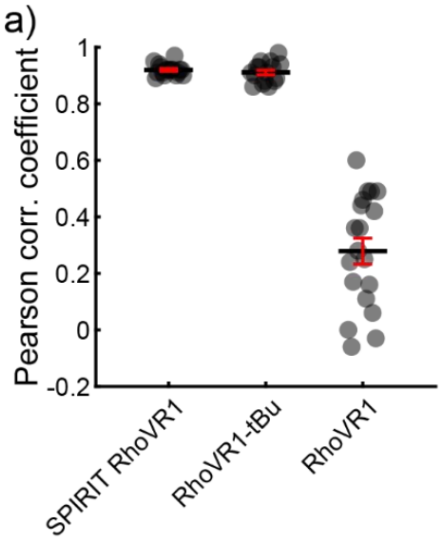






Both SPIRIT RhoVR 1 and RhoVR-tBu Localize to Mitochondria





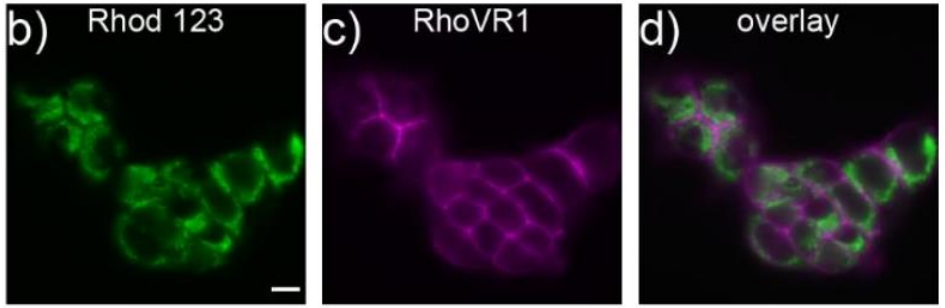
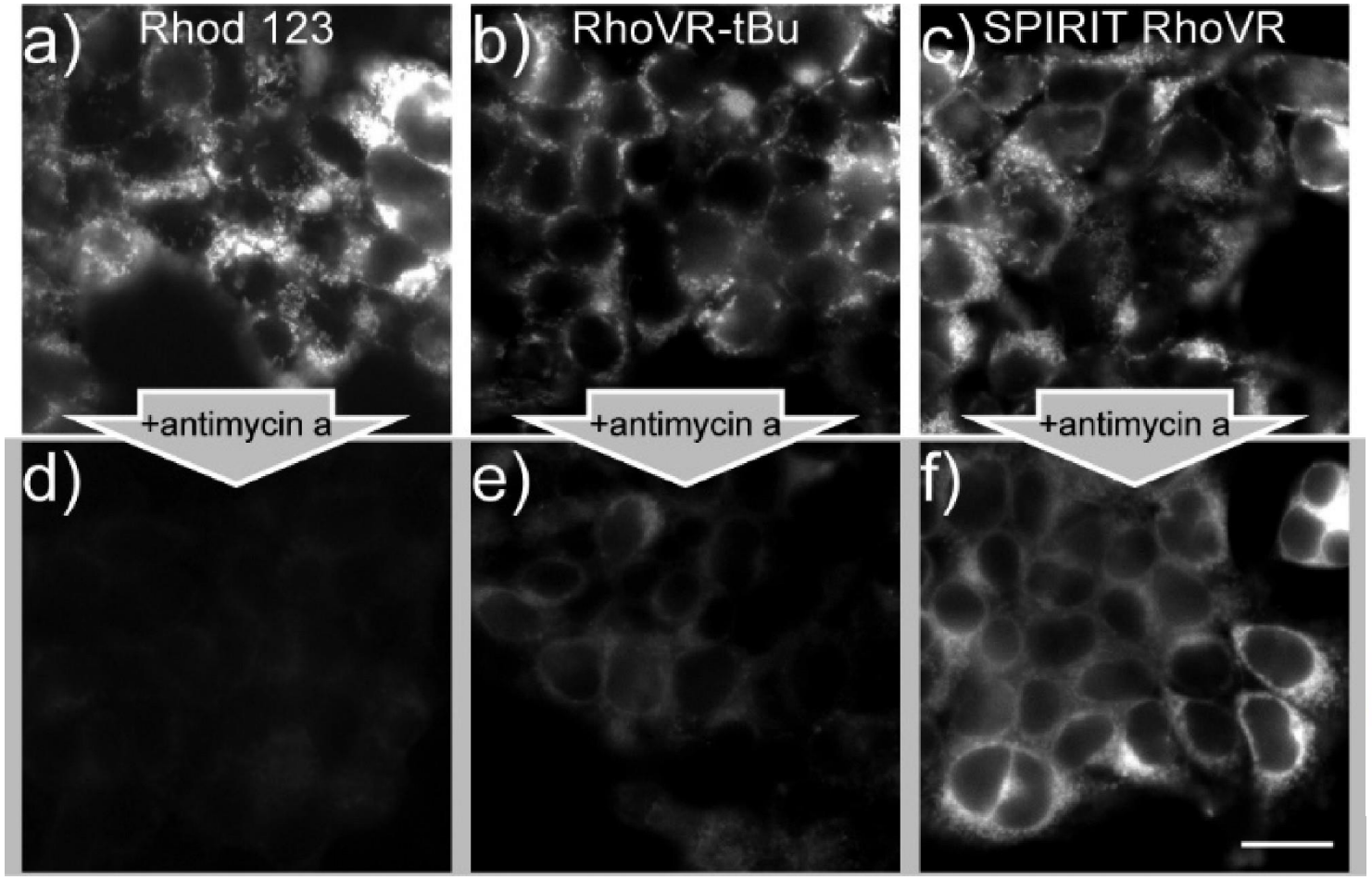


Figure 1. SPIRIT RhoVR 1 localizes to mitochondria in mammalian cells. Confocal fluorescence microscopy images of HEK cells stained with either (a) RhoVR-tBu (250 nM), (d) SPIRIT RhoVR 1 (250 nM), or (b, e) rhodamine 123 (250 nM). Overlay images of rhodamine 123 and either (c) RhoVR-tBu or (f) SPIRIT RhoVR 1. (g–i) Expanded views of the boxed region in panel e. Scale bar is 20 μm (a–f) or 10 μm (g–i).

SPIRIT RhoVR 1 remains localized to mitochondria following depolarization



抗霉素A：抑制复合物III，阻止电子转移到细胞色素c，消除H^+从基质向内膜空间的转移，导致ΔΨm的损失。

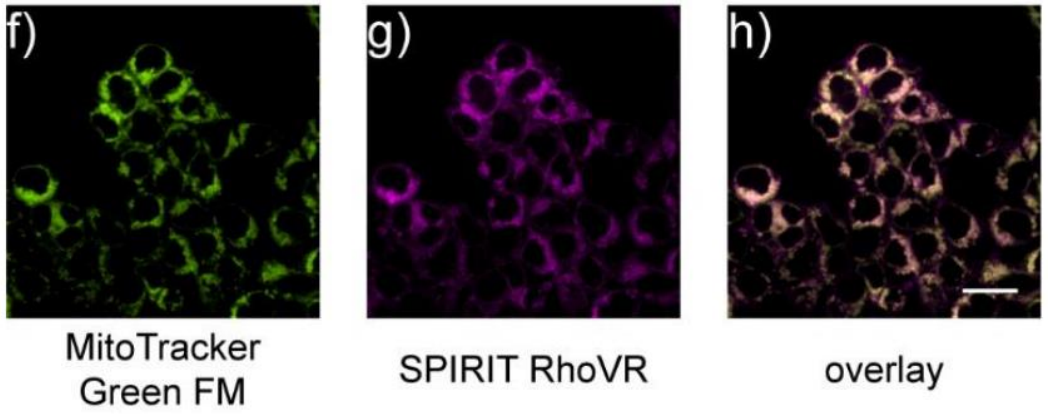


Figure 2. SPIRIT RhoVR1 is retained in mitochondria after dissipation of ΔΨm with antimycin A. Wide-field fluorescence microscopy of rhodamine or RhoVR derivative (250 nM) in the (a–c) absence or (d–f) presence of antimycin A (5 μg/mL). Wide-field fluorescence microscopy of SPIRIT RhoVR1 in the (c) absence or (d) presence of 5 μg/mL antimycin A for 90 min. Scale bar is 20 μm.

SPIRIT RhoVR 1 tracks depolarizations and hyperpolarizations in ΔΨm

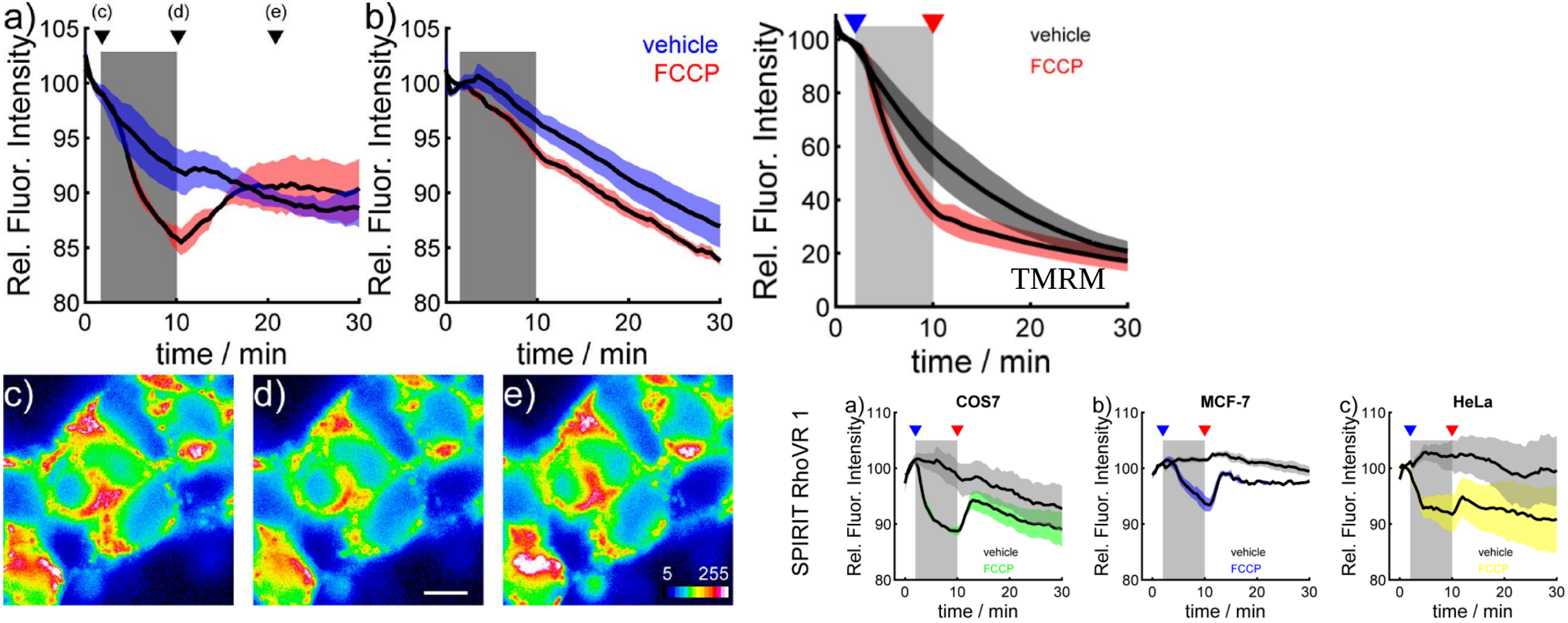


Figure 3. SPIRIT RhoVR 1 reports on ΔΨm dynamics in HEK cells. Plot of fluorescence intensity vs time for HEK cells stained with (a) SPIRIT RhoVR 1 (150 nM) or (b) SPIRIT RhoVR 0 (150 nM). At 2 min into the experiment (beginning of gray box), cells were perfused with either vehicle (ethanol, blue) or FCCP (500 nM, red). At 10 min (end of gray box), cells were perfused with HBSS. Data are mean (black line) ± SEM (colored shading) for three separate experiments. Representative pseudocolor images of SPIRIT RhoVR 1-loaded HEK cells (c) before, (d) during, and (e) after treatment with FCCP (500 nM). Scale bar is 10 μm for all images. Arrowheads in panel a indicate the time points of the representative images in panels c–e.

SPIRIT RhoVR 1 responds reversibly to changes in ΔΨm

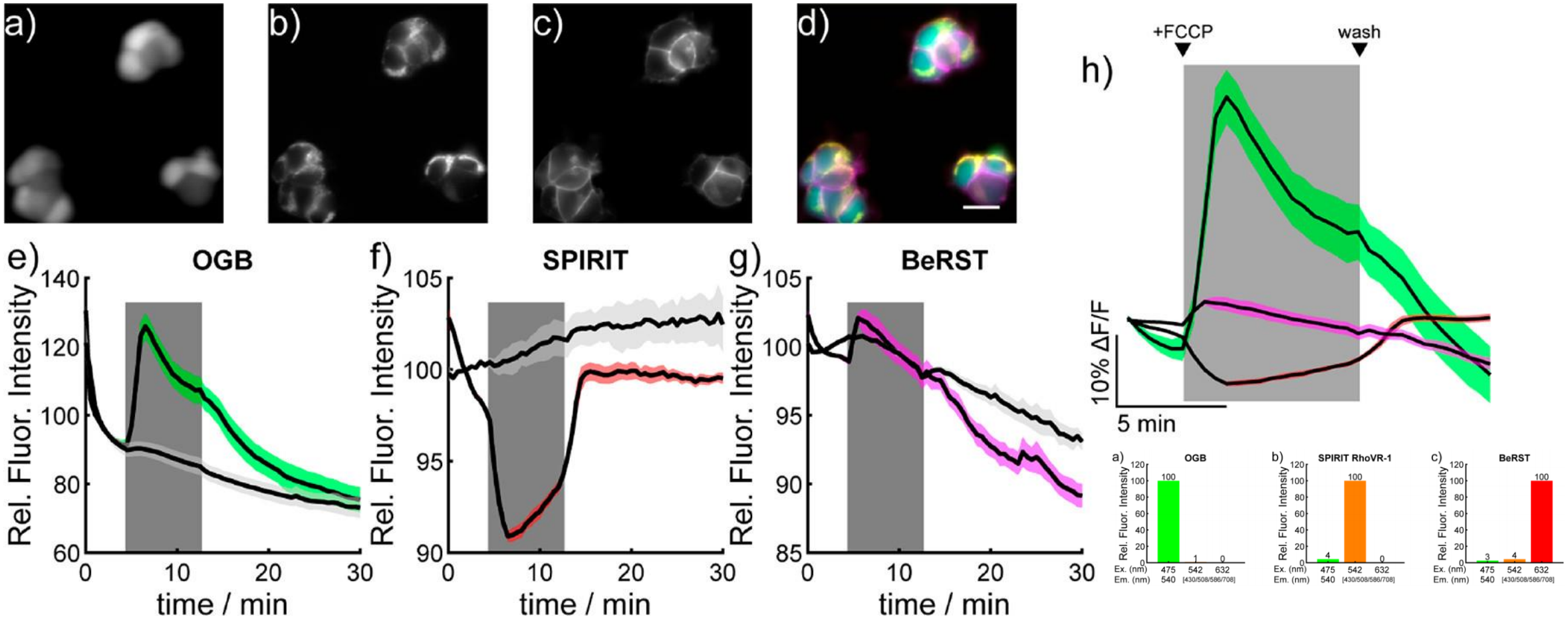


Figure 4. Simultaneous, multi-color imaging of mitochondrial membrane potential, cytosolic Ca^{2+}, and plasma membrane potential in mammalian cells. Wide-field epifluorescence images of HEK cells stained with (a) OGB (500 nM), (b) SPIRIT RhoVR 1 (150 nM), and (c) BeRST 1 (50 nM). (d) An overlay of the images showing cytosolic localization of OGB (green), mitochondrial localization of SPIRIT RhoVR 1 (yellow), and plasma membrane localization of BeRST (magenta). Scale bar is 20 μm. Plots of fluorescence vs time from HEK cells stained with (e) OGB, (f) SPIRIT RhoVR 1, or (g) BeRST. At 4 min into the experiment (beginning of gray box), cells were perfused with either vehicle (ethanol, light gray) or FCCP (500 nM, colored trace). At 4 min (end of gray box), cells were recovered by perfusion with HBSS. Data are mean (black line) ± SEM (colored shading) for three separate experiments. (h) Zoomed-in plot of the response of OGB (green), SPIRIT RhoVR 1 (red), and BeRST 1 (magenta). The gray box indicates the start and end of FCCP perfusion.