A brief introduction to pKa
&
pH Fluorescent Probe

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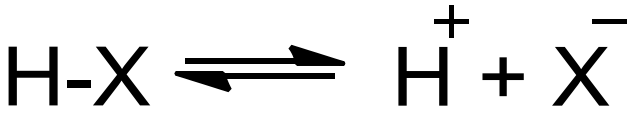
pKa

- Acidities are quantified by pKa values. The pKa of an acid HX is defined as



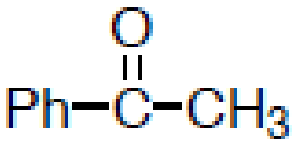
$$pK_a = -\log\left(\frac{[H^+][X^-]}{[HX]}\right)$$

- The larger the pKa, the less acidic the compound.

Factors

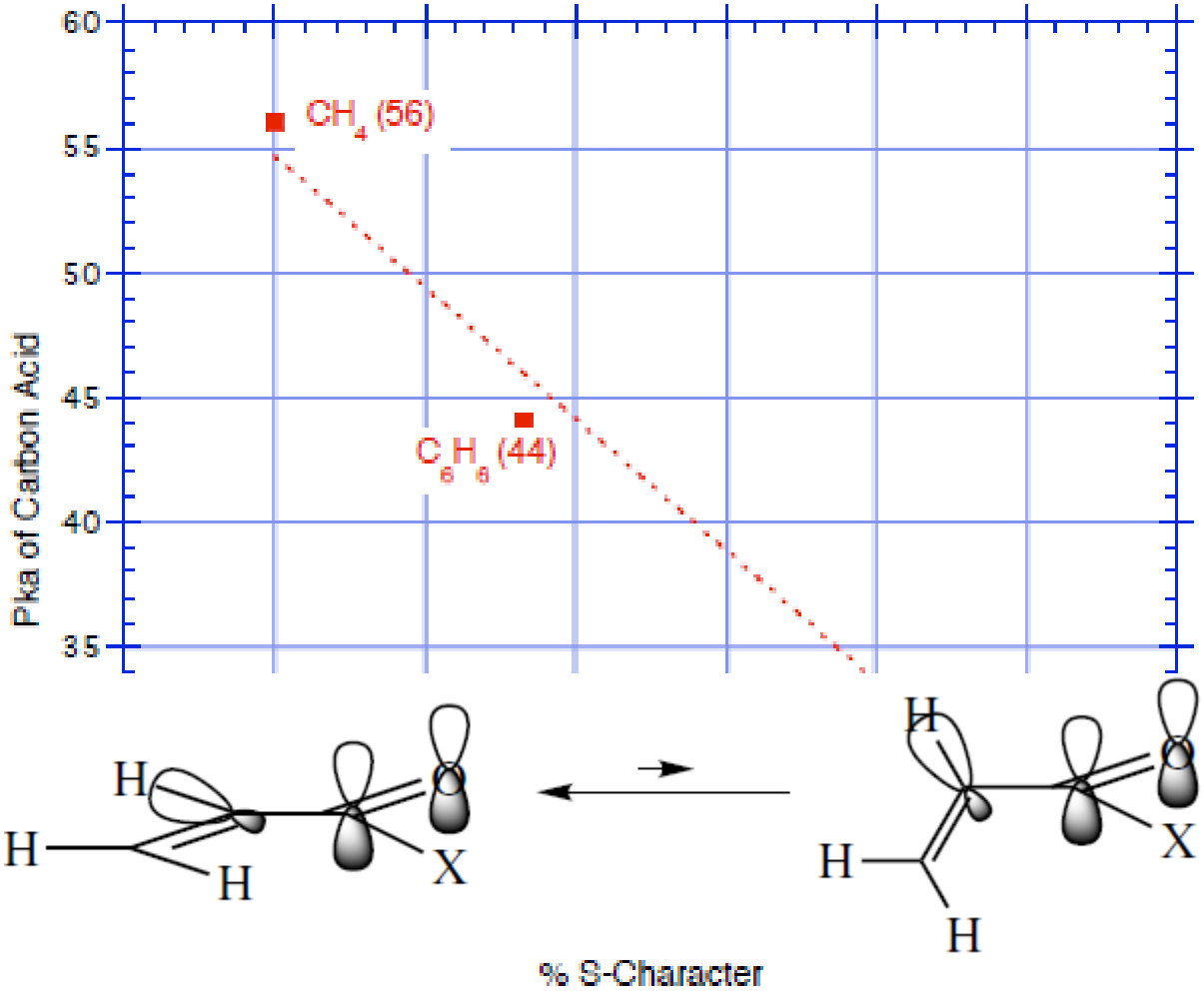
- Medium Effects
- Hybridization Effects
- Substituent Effects

Medium Effects

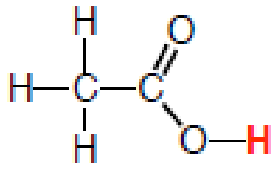
Substrate	DMSO	HOH	Δ pKa
HOH	31.2	15.7	15.5
HSH	14.7	7.0	7.7
MeOH	29.0	15.3	13.7
C_6H_5OH	18.0	9.9	8.1
$O_2N–CH_3$	17.2	10.0	7.2
	24.6	17	7.6

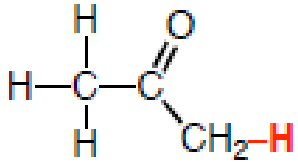
Hybridization Effects

There is a direct relationship between %S character & hydrocarbon acidity

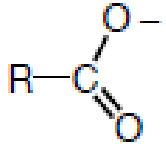


Substituent Effects

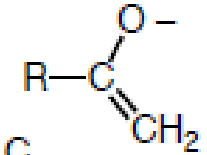


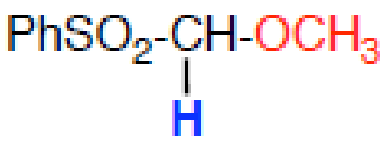
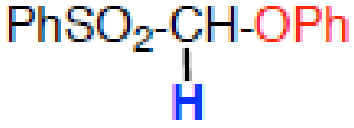
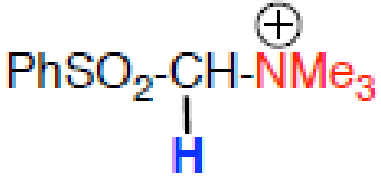


(DMSO) $pK_A = 12.3$ $pK_A \approx 26.5$



Carboxylate ion more stabile than enolate because O more electronegative than C



	pK_A (DMSO)	
	30.7	Inductive Stabilization versus Lone Pair Repulsion (-I vs +M -Effect)
	27.9	
	19.4	Inductive Stabilization

Measurement

Sirius T3理化常数pKa logP/D测量仪

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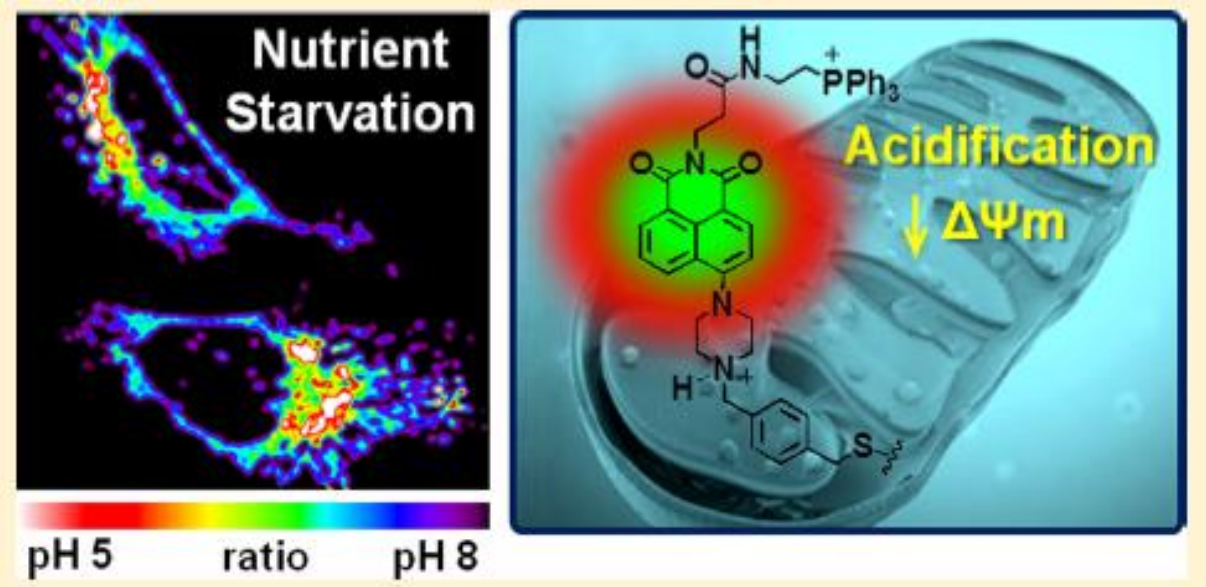
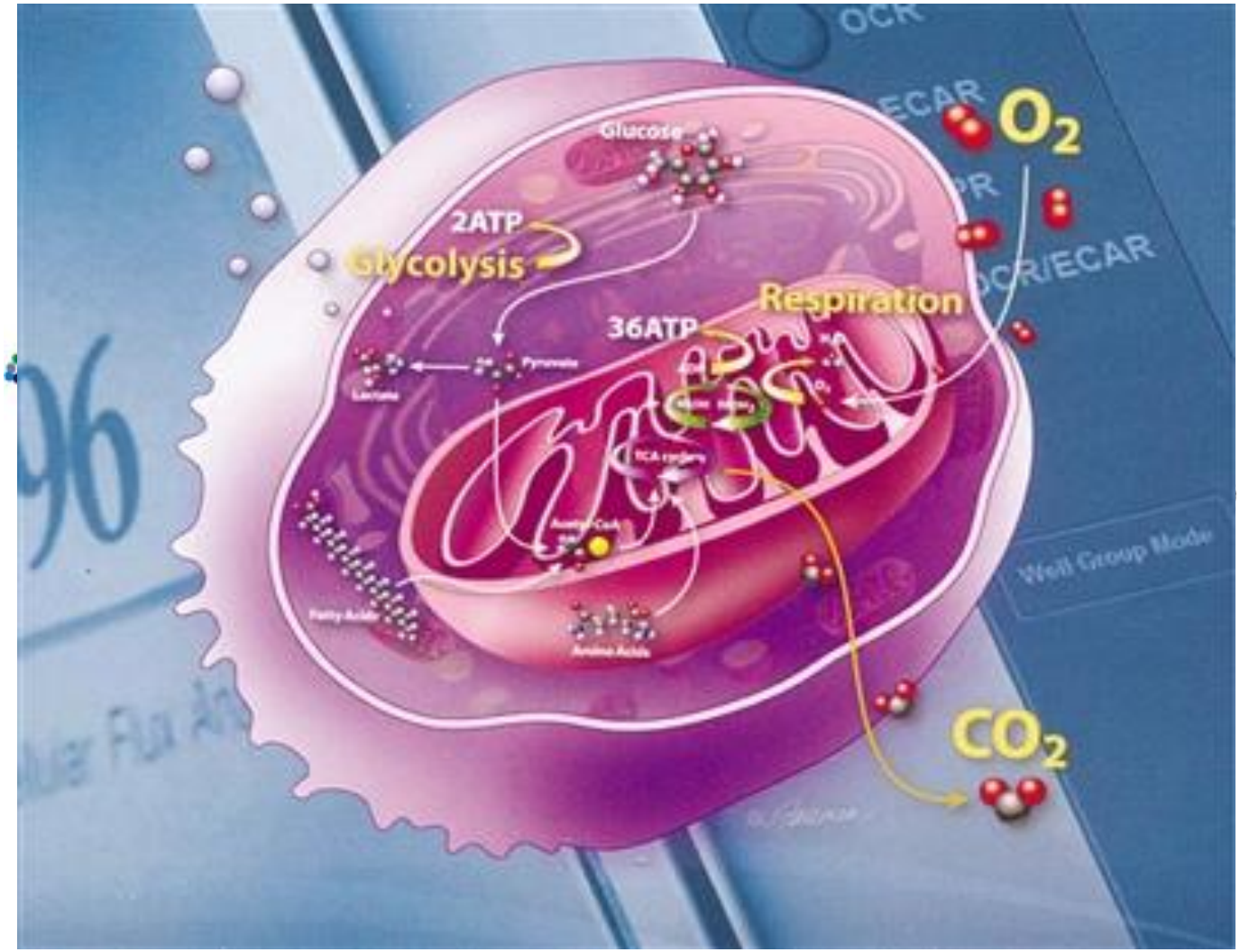
Mitochondria-Immobilized pH-Sensitive Off–On Fluorescent Probe

Min Hee Lee, Nayoung Park, Chunsik Yi, Ji Hye Han, Ji Hye Hong, Kwang Pyo Kim,Dong Hoon Kang, Jonathan L. Sessler, Chulhun Kang, and Jong Seung Kim

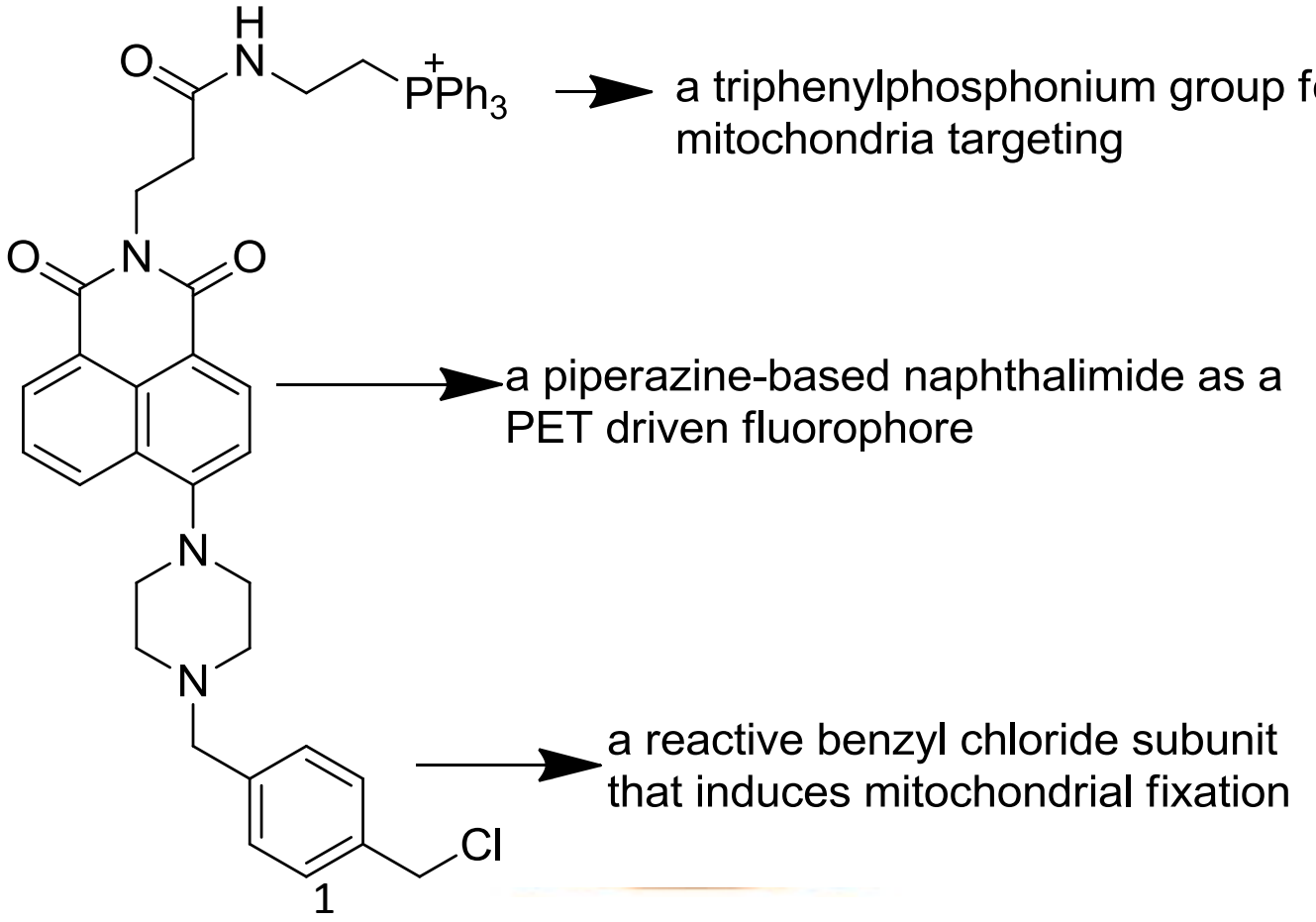


Background

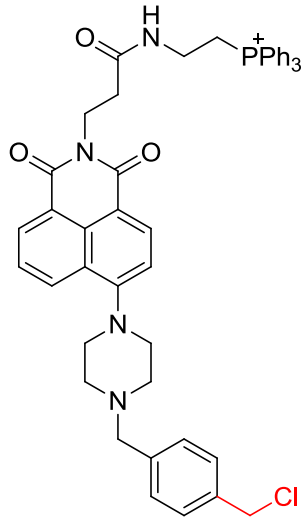


Youle, R. J.; Narendra, D. P. Mol. *Cell. Biol.* 2011, 12, 9−14.
Kim, I.; Rodriguez-Enriquez, S.; Lemasters, J. J. Arch. *Biochem.Biophys*. 2007, 462, 245−253.

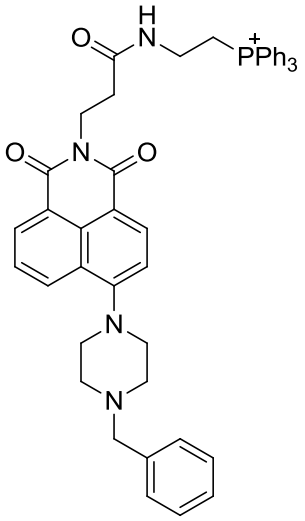
pH sensing mechanism



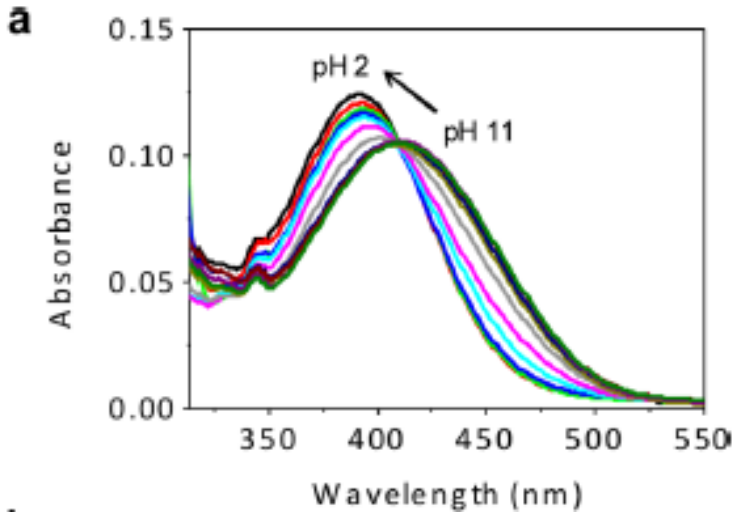
In vitro

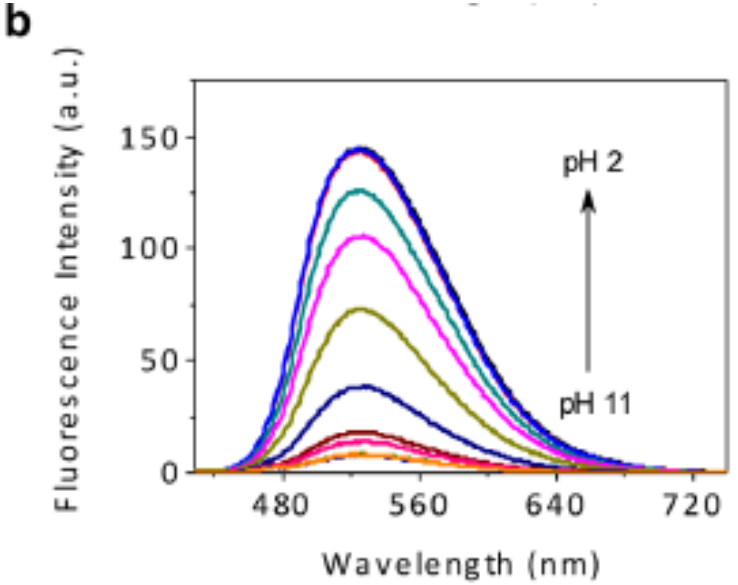

H
N
O
O
N
O
N
N
PPh3
Cl

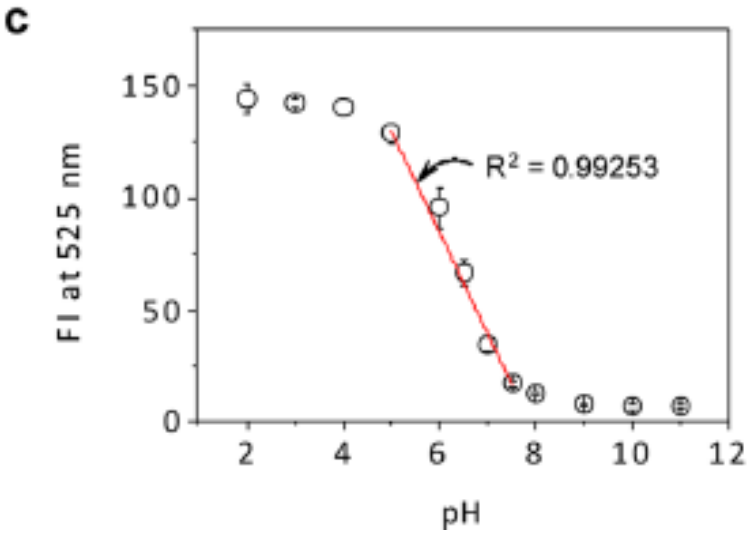
1


H
N
O
O
N
O
N
N
PPh3

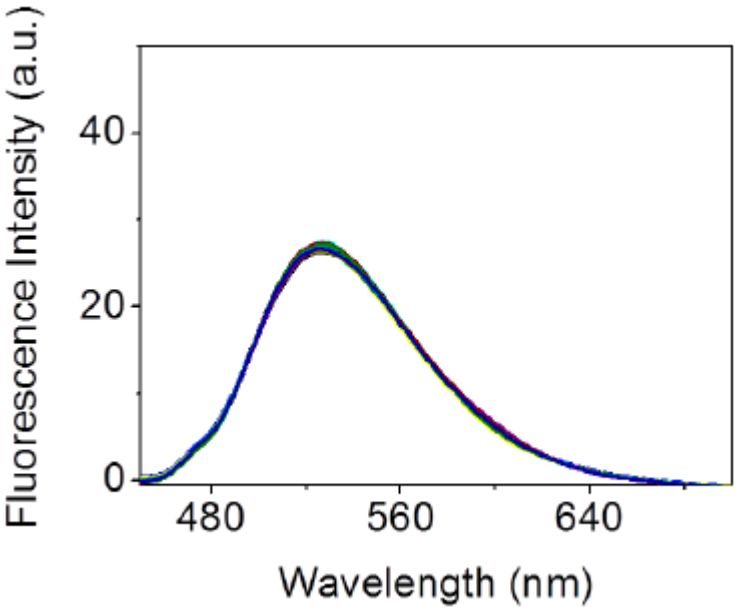
2


a
0.15
0.10
0.05
0.00
Absorbance
pH 2
pH 11
350
400
450
500
550
Wavelength (nm)

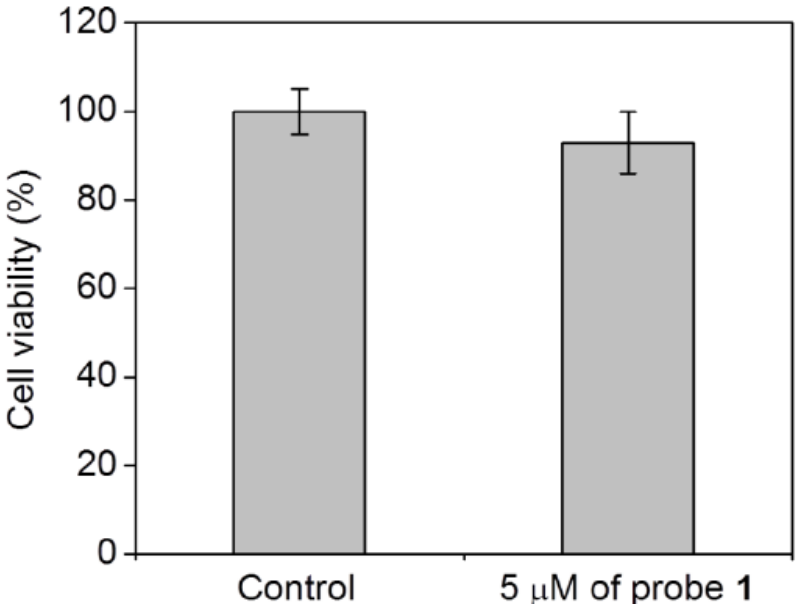

b
Fluorescence Intensity (a.u.)
150
100
50
0
pH 2
pH 11
480
560
640
720
Wavelength (nm)


c
FI at 525 nm
150
100
50
0
R² = 0.99253
2
4
6
8
10
12
pH

Stability and MTT

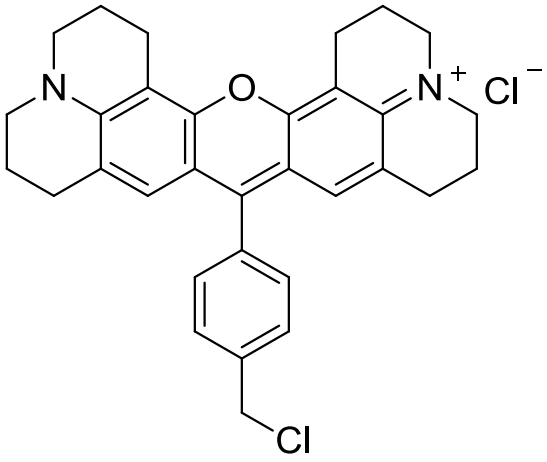


Na+, K+, Ca2+, Zn2+, Mg2+, Mn2+, Cu2+, Fe2+, Fe3+, thiols (GSH, Cys, Hcy)



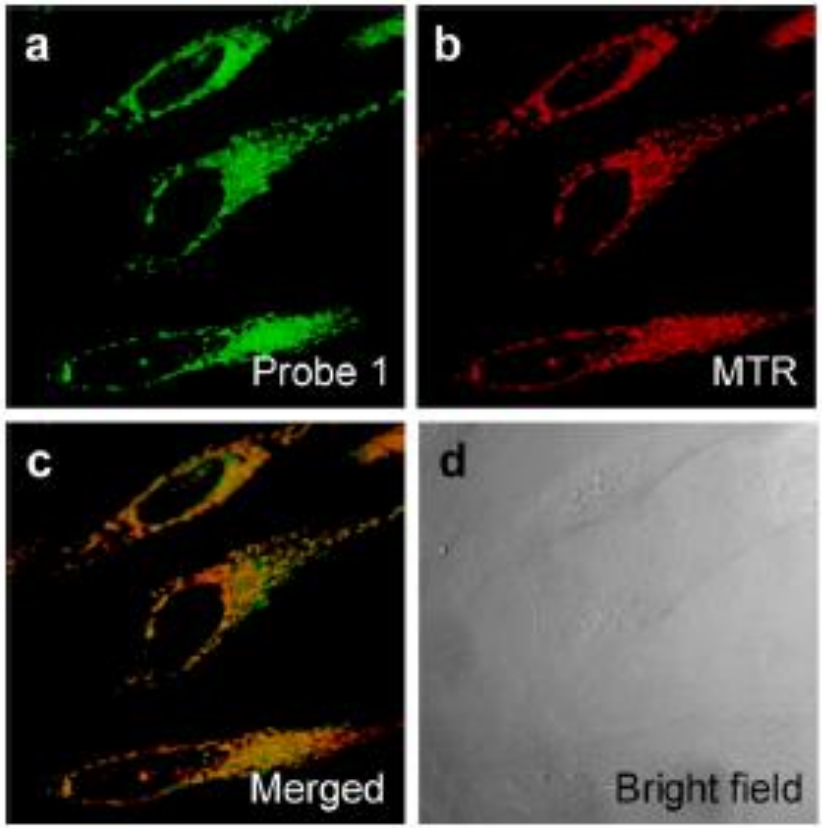
MTT assay

in vivo--selectivity

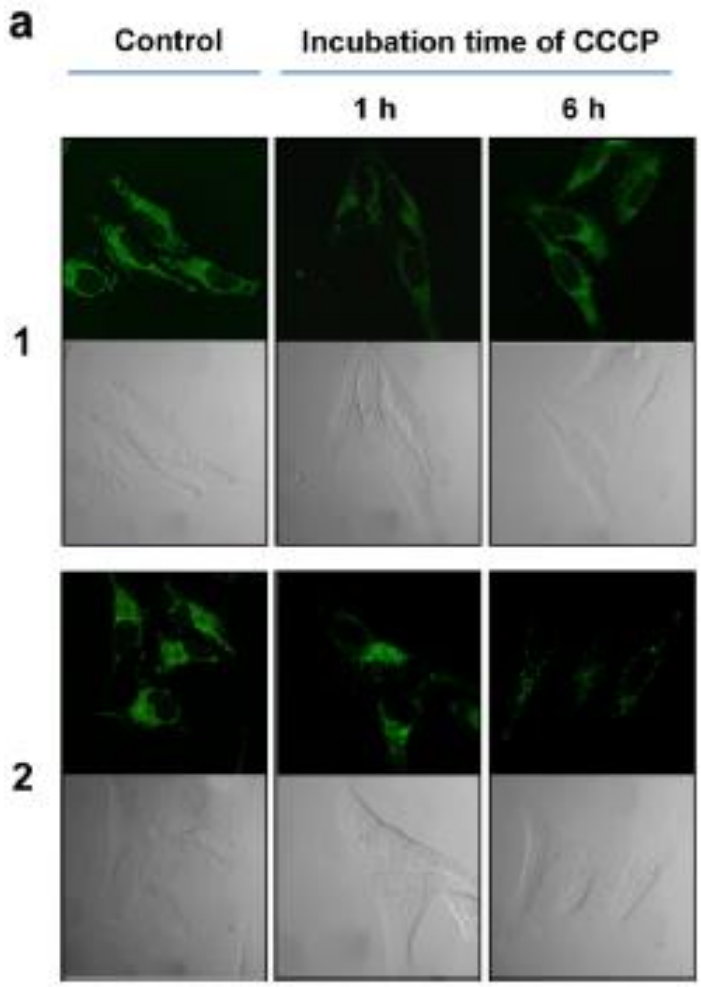


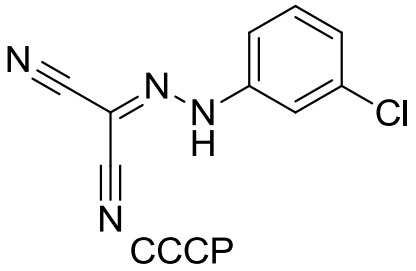
MitoTracker Red(MTR)

mitochondrion-specific fluorescent probe

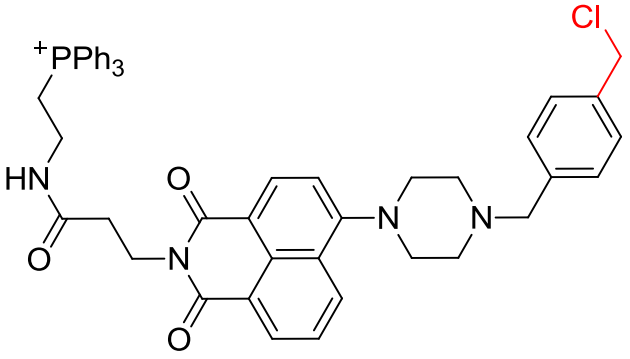


Immobilization

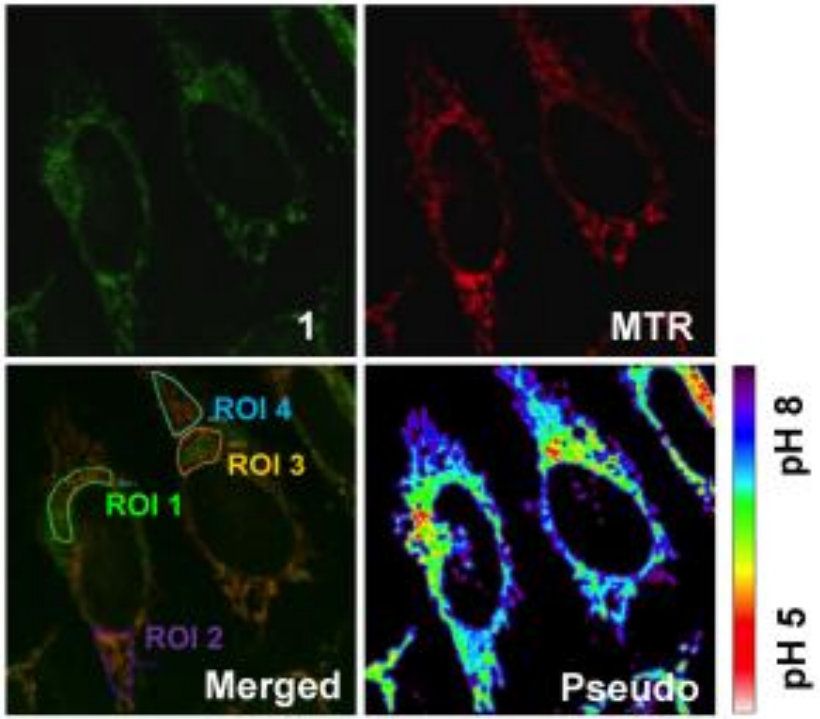




CCCP induces an uncoupling of the mitochondrial membrane potential (Δψm)

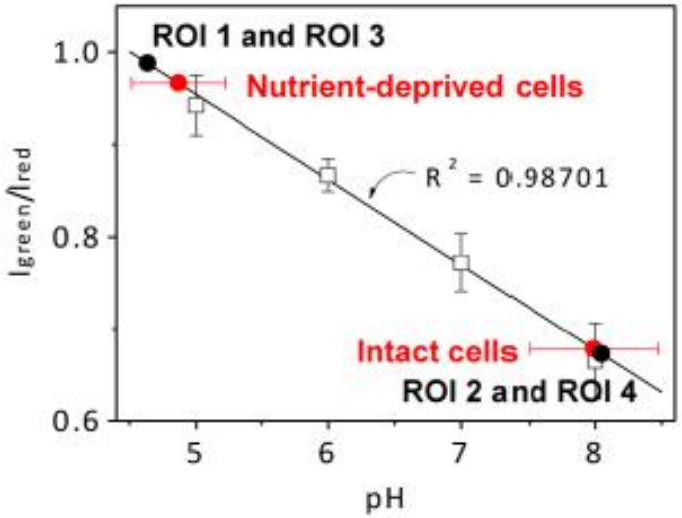


c



Area	I_{green}/I_{red}	pH
Pseudo red-green color (ROI 1 and ROI 3)	0.988085	4.63
Pseudo blue-purple color (ROI 2 and ROI 4)	0.672852	8.05

b

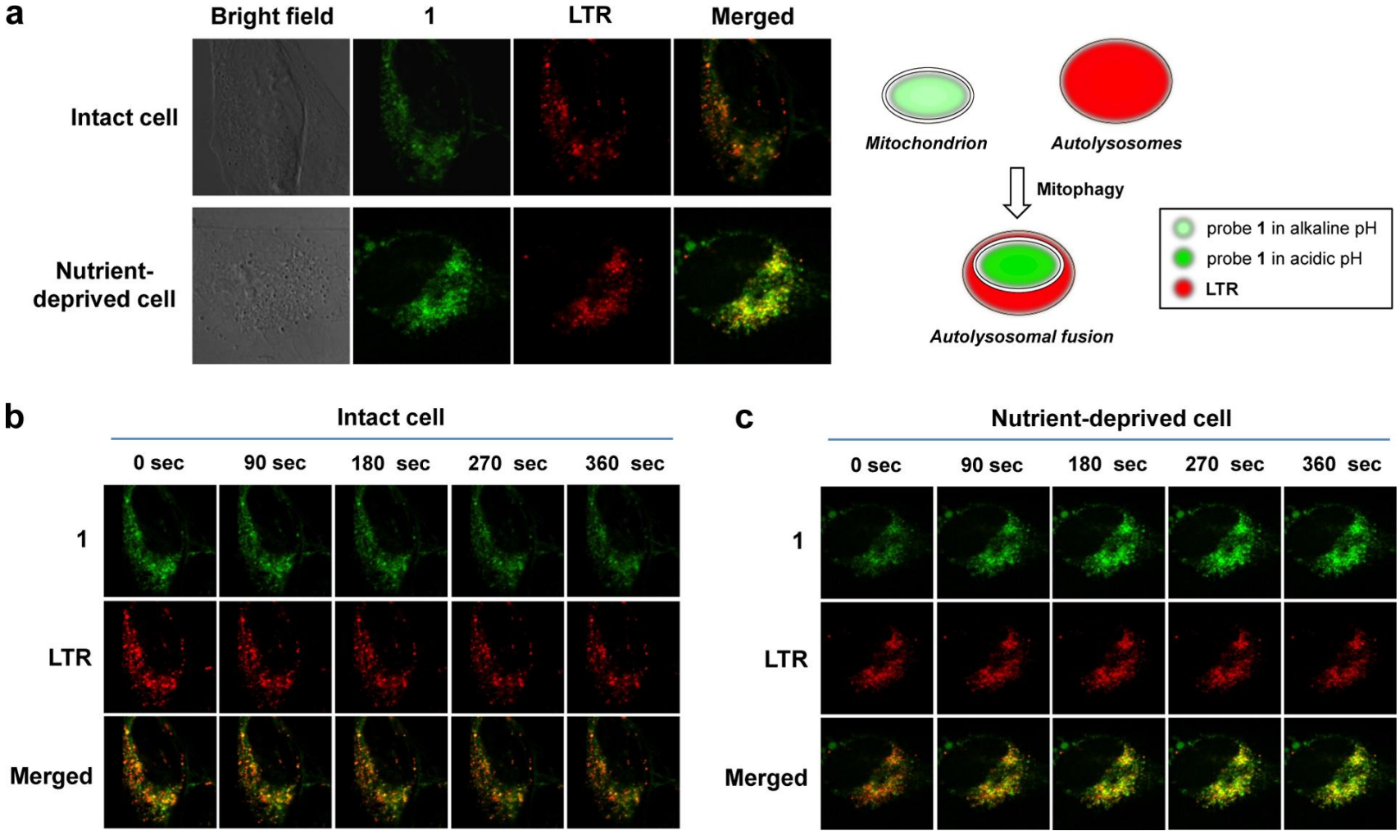


HeLa cell	I_{green}/I_{red}	pH
Intact cell (n = 5 cells)	0.678283	7.99 (±0.49)
Nutrient-deprived cell (n = 8 cells)	0.966665	4.87 (±0.35)

Real-time monitoring

LysoTracker Red(LTR)

Pepstatin A is a protease inhibitor and was used to delay mitochondrial degradation by proteases in the autolysosomes.



Summary

- The probe allow the direct and reliable mitochondrial pH measurement in whole cells.
- Achieve the real time monitoring of pH changes associated with the mitochondrial acidification and fusion.
- Act a means of distinguishing between physiological and pathological states or screening potential new mitochondria-targeting drugs.

Thank you !