Chemical sensor for quantification of GSH in cell

Literature seminar #2

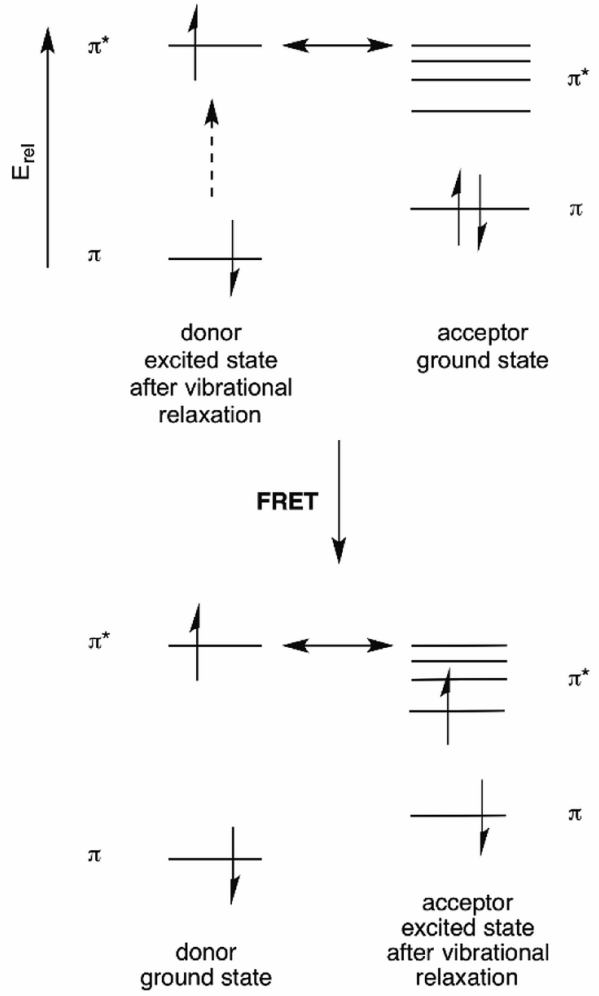
2023/08/10

M1 Shuhei Terada

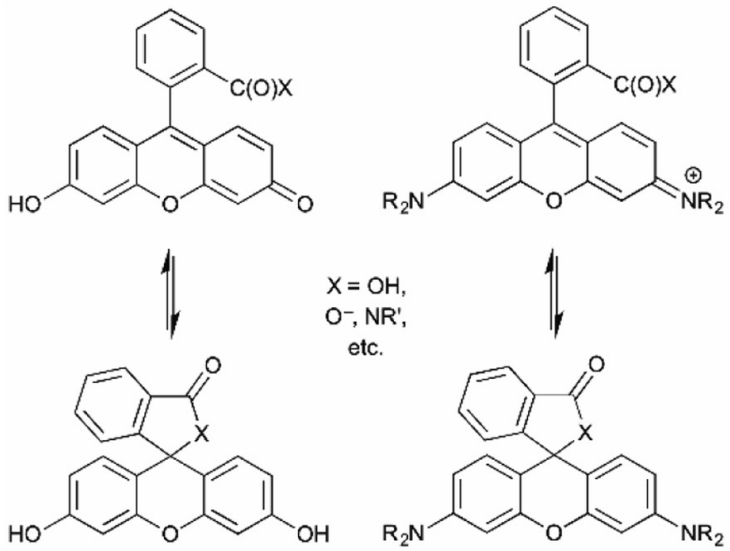
Contents

Fluorescence fundamentals

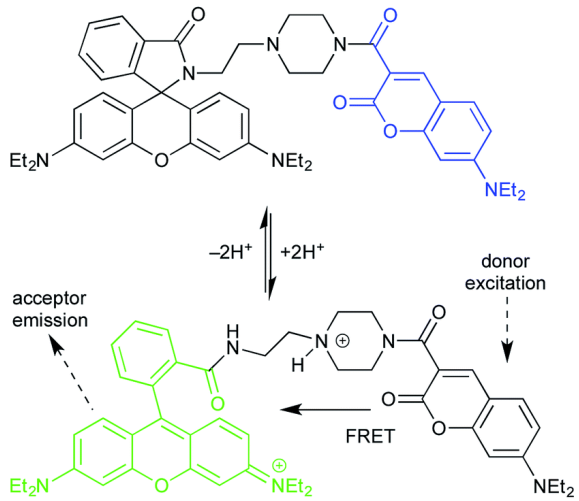
FRET (Förster resonance energy transfer)



Intramolecular spirocyclization



e.g.
FRET-based probe for measurement of pH in lysosomes.



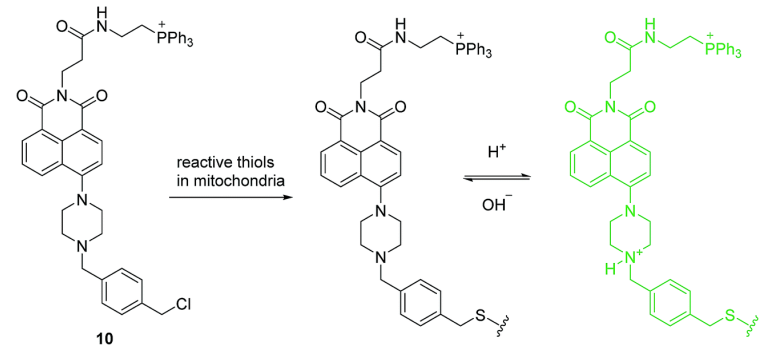
Photoinduced electron transfer (PeT)
Internal charge transfer (ICT)
etc.

✓Fluorescent probes use the basis of fluorescence to control fluorescence switching.

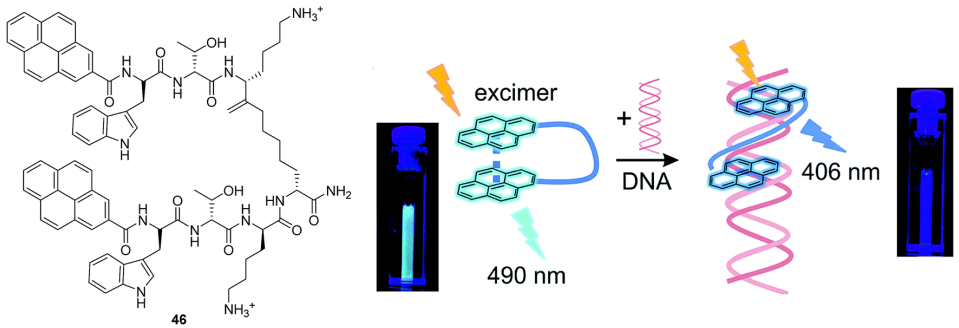
Fu, Y. and Finney, N. *RSC. Adv.* **2018**, 8, 29051-29061

Targets of chemical sensors

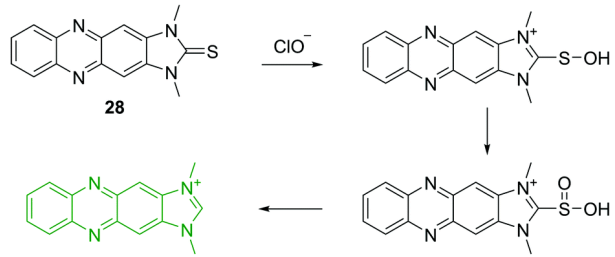
- Cations: H^+, Mg^{2+}, Cu^{2+}, etc.
- Anions: Pyrophosphate (PPi), $O_2^{-\bullet}$, ClO^-, etc.
- Small neutral molecules: GSH, H_2O_2, NO, etc.
- Biomacromolecules: DNA, Aβ plaques, β-Galactosidase, etc.



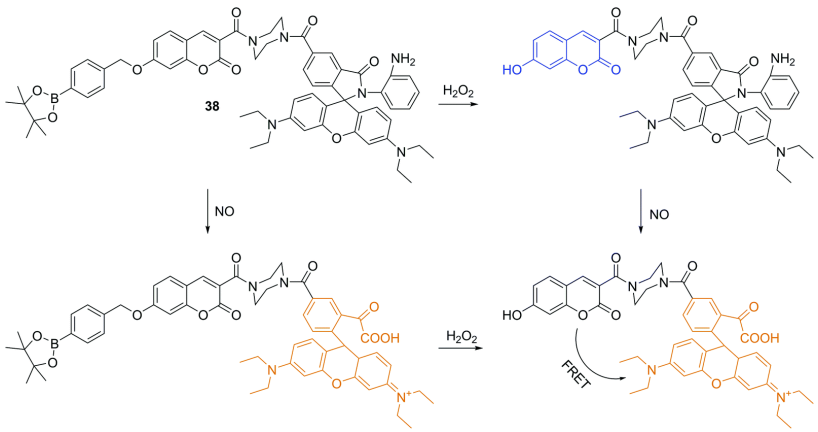
Detection of pH in mitochondria



Interaction with nucleic acid



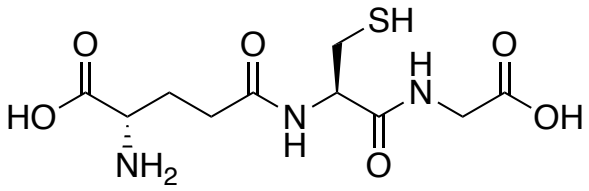
Detection of ClO^-



Detection of H_2O_2 and NO

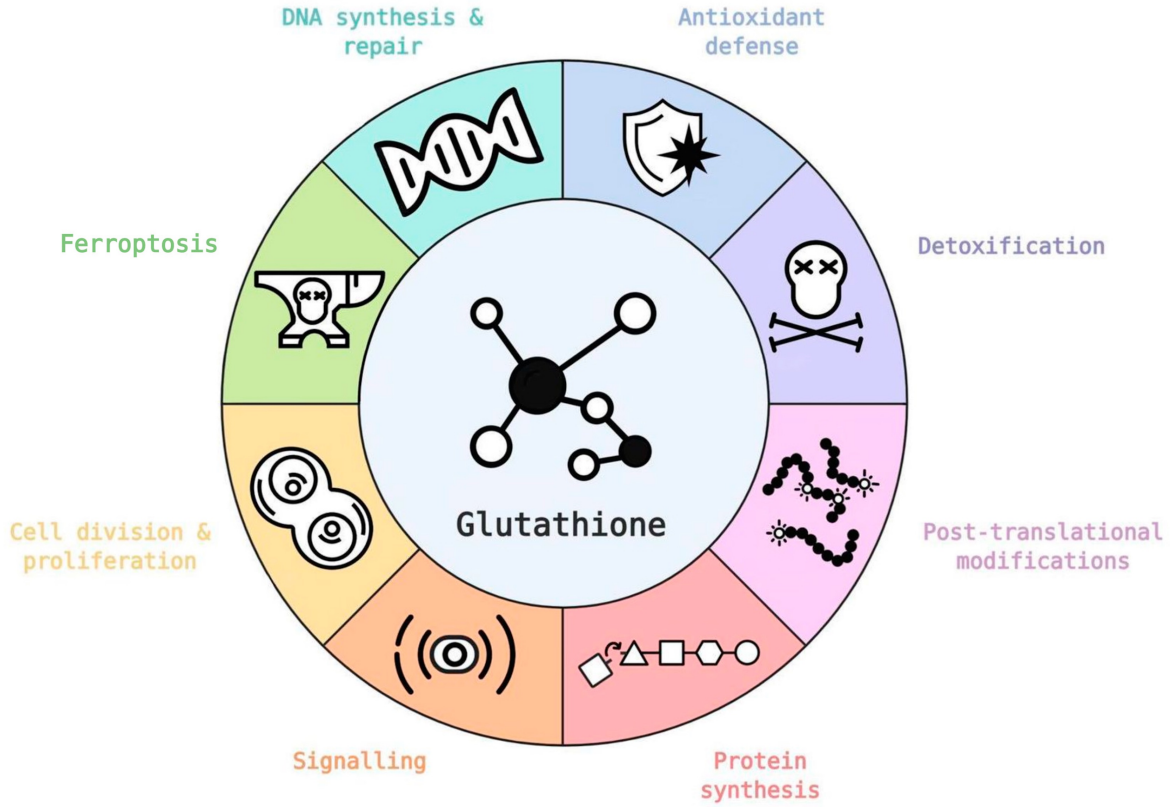
James, T. et al. *Chem. Soc. Rev.* **2017**, 46(23), 7105-7123

Glutathione in cell



Glutathione (GSH)
(γ-glutamyl-cysteinyl-glycine)

- The most abundant low-MW cysteine-containing thiol in cell (0.5-10 mM)
- Critical role in a cellular redox state
- The ratio of GSH/GSSG is an important indicator.
- Alterations in GSH synthesis, metabolism and homeostasis are associated with diseases: Parkinson's disease, immune dysfunctions, many type of cancer, etc.

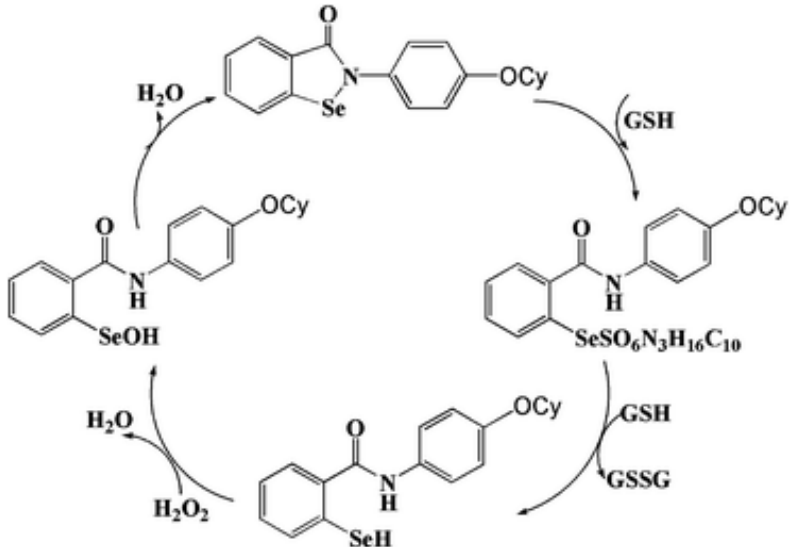


✓ Real-time imaging and quantification of intracellular GSH dynamics in living cells would provide important pathophysiological insights.

Luke. K, et al, *Biomolecules*, **2020**, 10(10): 1429

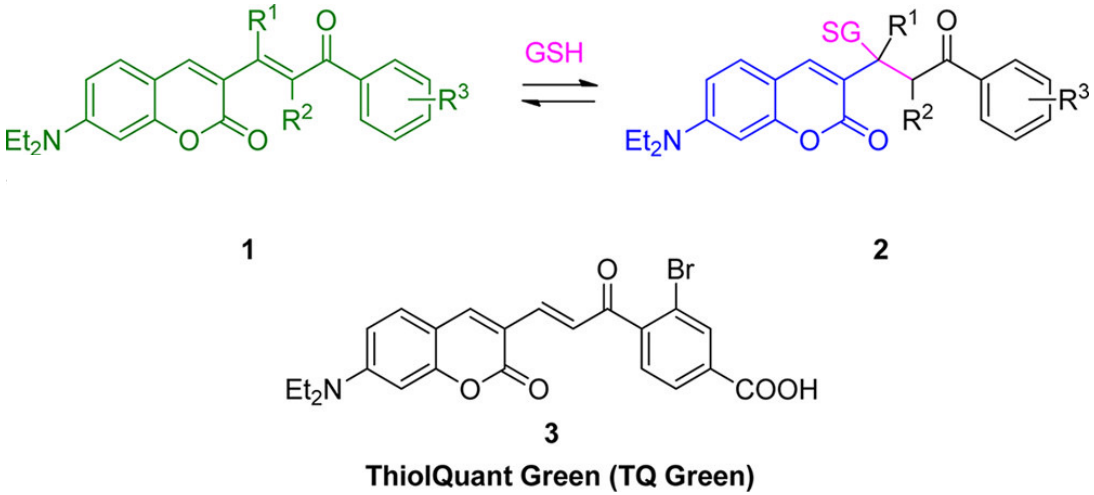
Conventional fluorescent probe for GSH imaging

GSH and H_2O_2 detection using CyO-ebselen



- ○ Reversible reaction
- ✖ Slow reaction
- ✖ Response range is not optimized to intracellular GSH concentration

Intramolecular spirocyclization (TQ-Green)



- ○ Reversible reaction
- ✖ Slow reaction (k = 0.144 M^{-1} s^{-1})
- ✖ Low quantum yield (< 0.01 in PBS)

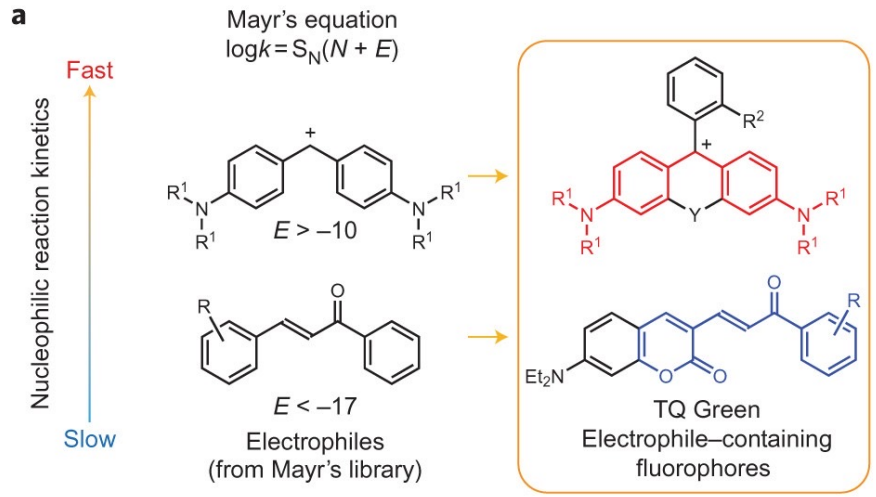
Tang. B, et al, *Chem. Sci.* **2013**, 4(3), 1079-1086

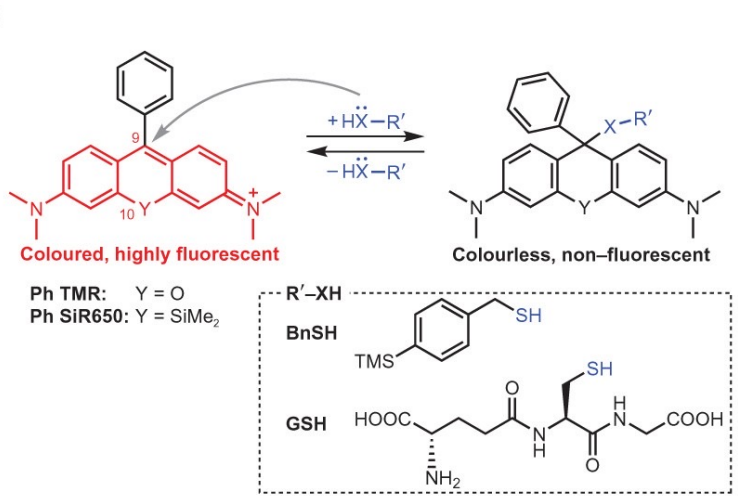
Wang, J. et al, *ACS. Chem. Biol.* **2015**, 10, 3, 864-874

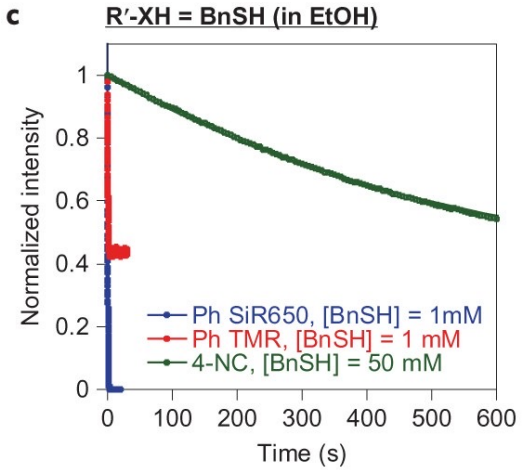
Molecular design of rapidly responsive and reversible fluorescent probes for GSH

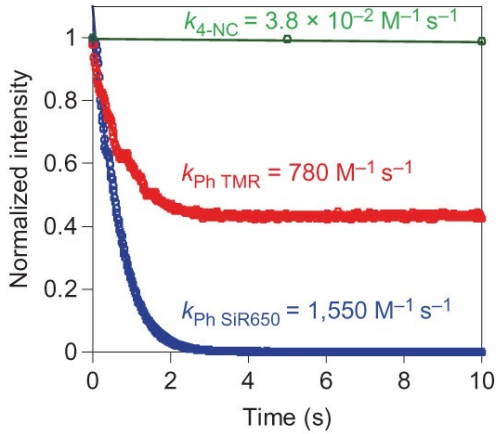
Main

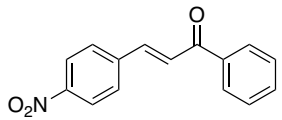
Problems to be solved
1. Rapid reaction
2. Reversibility
3. Quantum yield











4-NC
(4-nitrochalcone)

✓Si-rhodamine as a candidate for rapid and reversible detection of GSH
✓Benzhydrylium ion reacts faster with nucleophile than α,β-unsaturated ketone.

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

Molecular design of rapidly responsive and reversible fluorescent probes for GSH

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1. Rapid reaction
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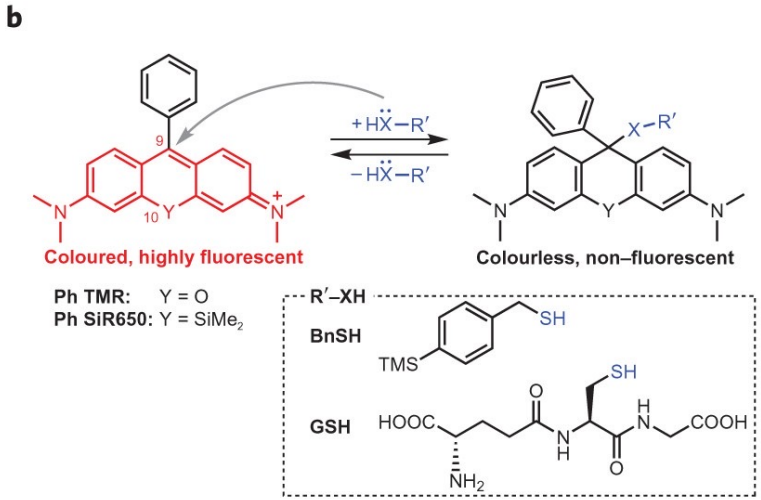

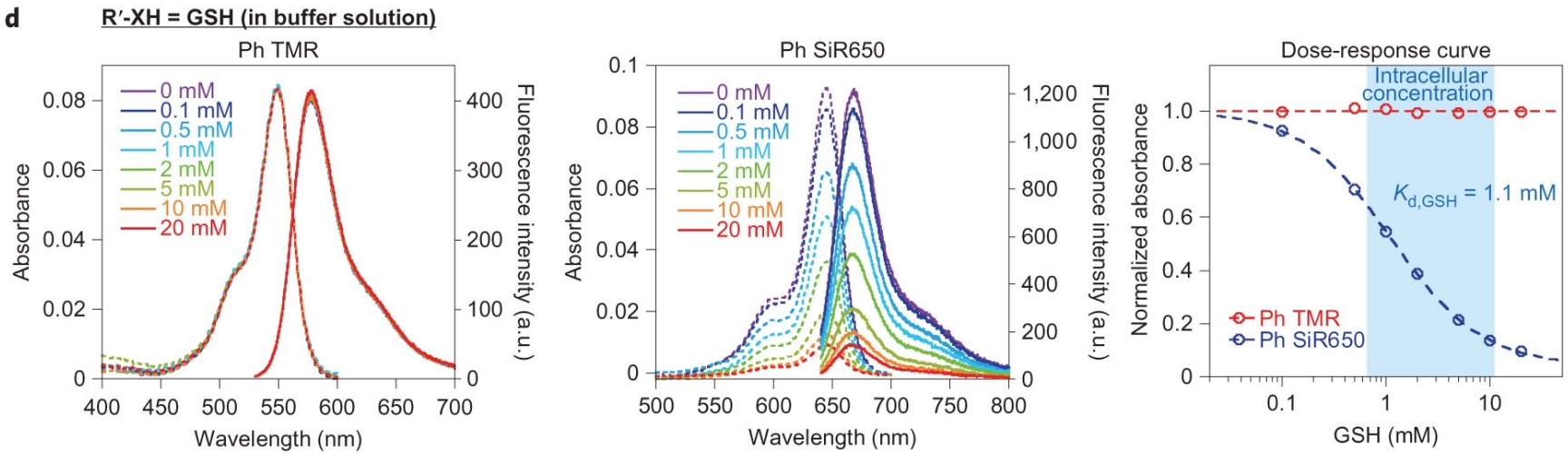

✓Ph SiR650 showed spectral changes upon addition of GSH (0-20mM).

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

Molecular design of rapidly responsive and reversible fluorescent probes for GSH

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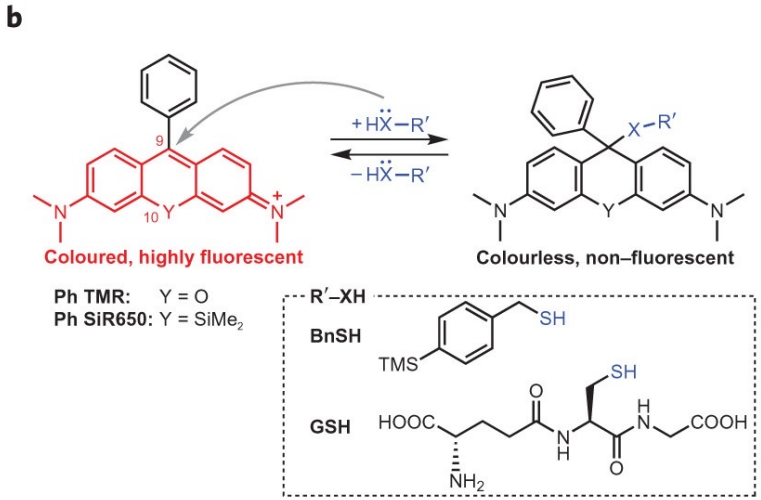
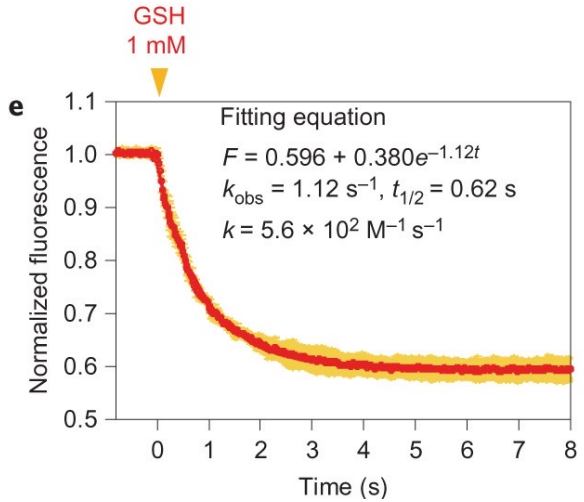
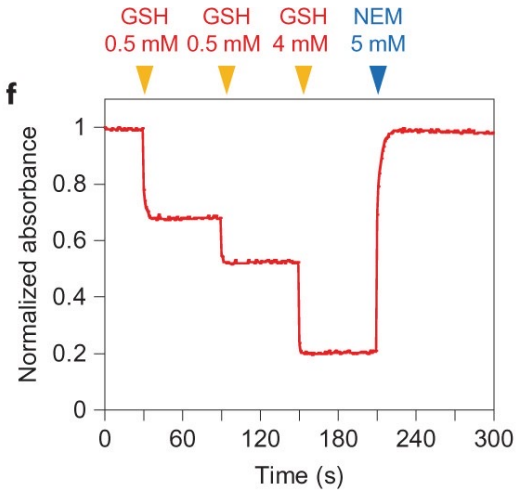
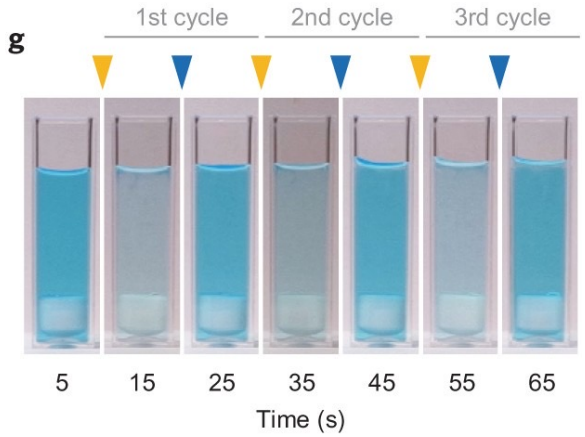
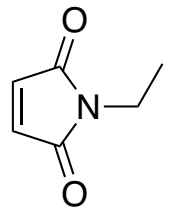










NEM
(N-ethylmaleimide)
An irreversible thiol scavenger

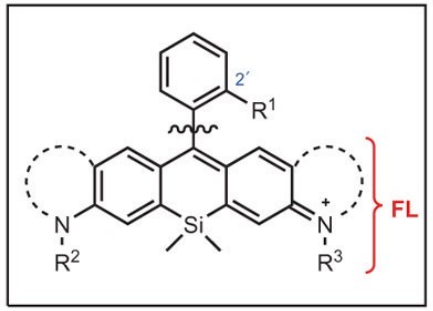
✓Ph SiR650 showed Time-dependent fluorescence decay upon addition of GSH.

✓Fluorescence decay of Ph SiR650 was reversible.

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

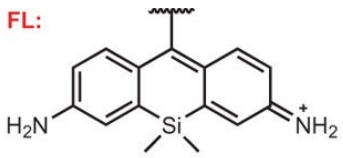
Optimization of SiR structure

Main

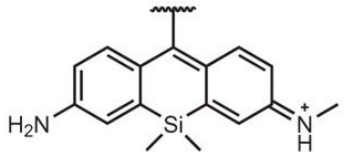


R^1 = H, F, Cl, Me, CF_3

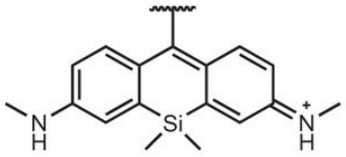
FL:



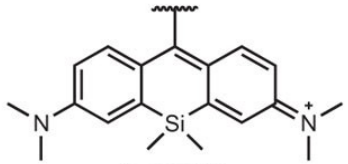
A: SiR600



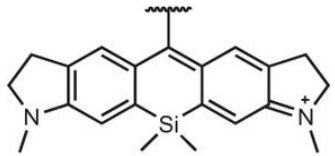
B: SiR610



C: SiR620



D: SiR650



E: SiR700

	R^1	FL	$\lambda_{abs,max}$ (nm)	$\lambda_{fl,max}$ (nm)	QY†	$K_{d,GSH}$ (mM)‡	LUMO level§ (hartrees)
1	H	D	645	668	0.14	1.1	
2	F	D	653	676	0.19	1.3	
3	Cl	D	655	676	0.30	27	
4	Me	D	646	667	0.31	>100	−0.21784
5	CF_3	D	655	678	0.33	n.d.‖	
6	Me	A	593	613	0.38	1.0	−0.22940
7	Me	B	604	627	0.31	7.9	−0.22531
8	Me	C	618	638	0.43	80	−0.22144
9	Me	E	691	720	0.13	n.d.	−0.20939

Problems to be solved
1. Rapid reaction
2. Reversibility
3. Quantum yield

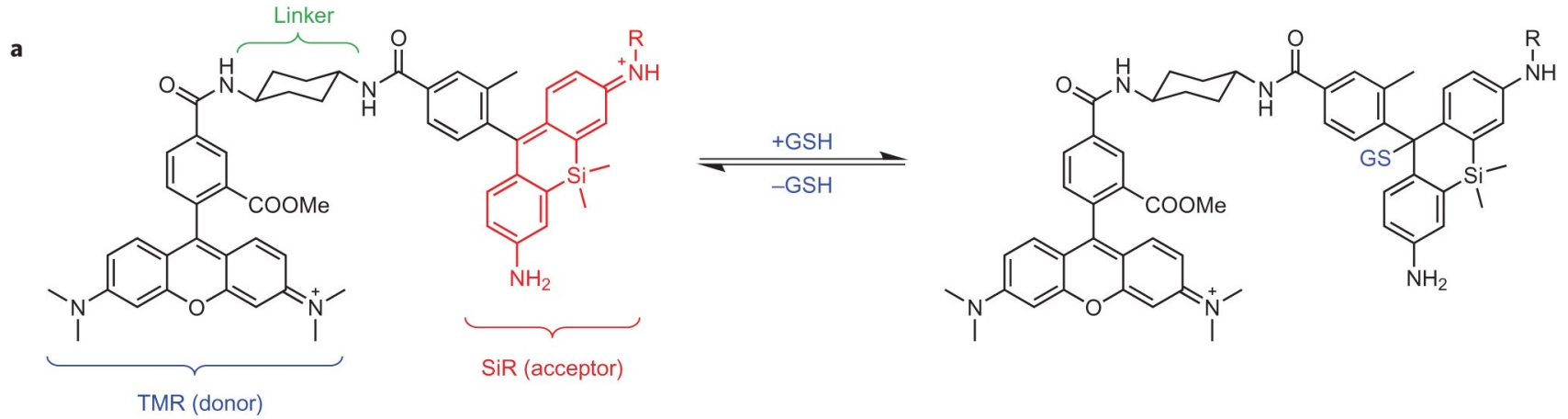
*Measured in 0.2 M sodium phosphate buffer (pH 7.4 containing 1% DMSO)
†Absolute quantum yield; ‡Determined from absorption spectra
§LUMO energy level of the corresponding xanthene moiety. Data were calculated at the B3LYP/6-31+G* level with Gaussian 09
‖n.d., not determined (>100 mM).

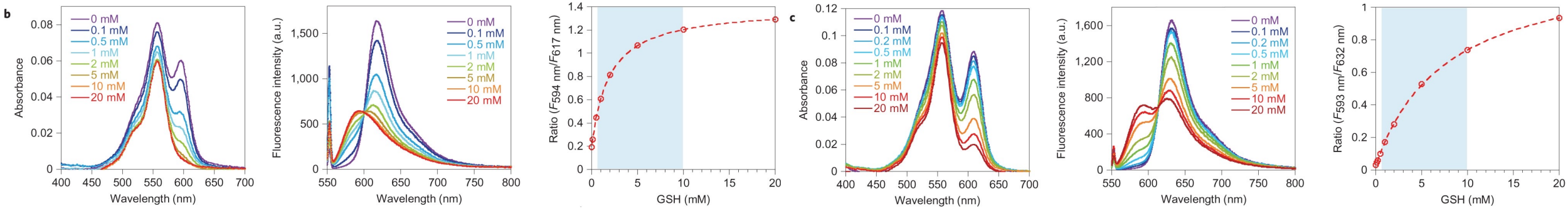
✓Optimization of substituents improved quantum yields.

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

FRET-based ratiometric probes for quantitative GSH imaging

Main





QG0.6

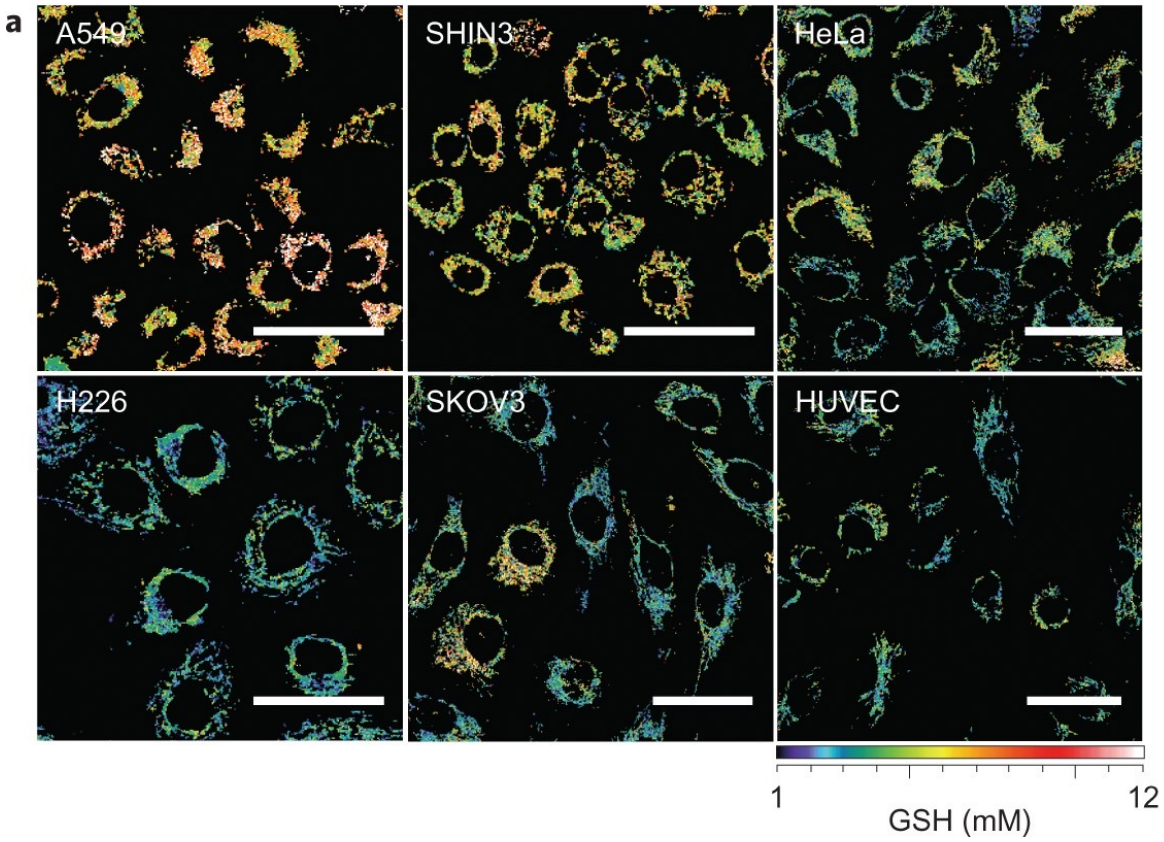
QG3.0

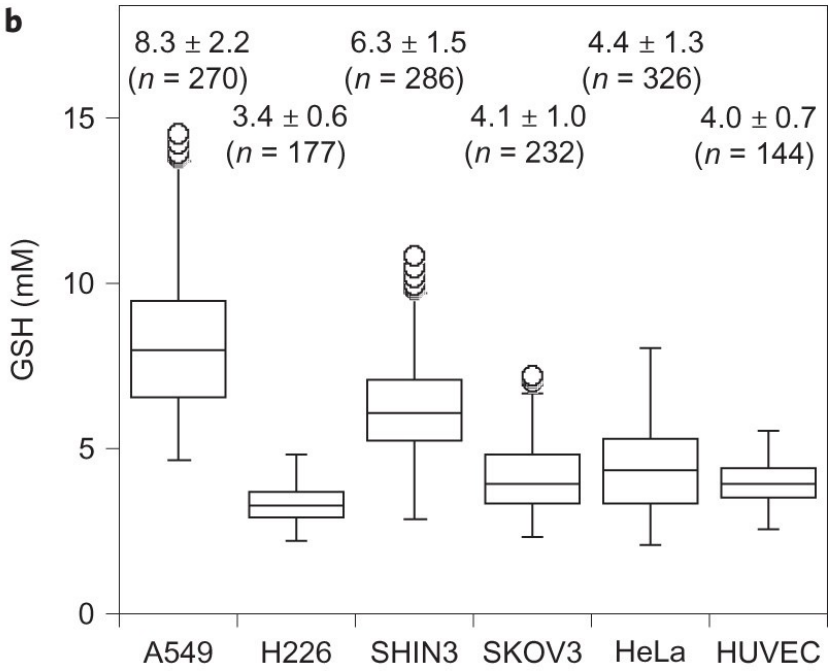
✓TMR: donor for the FRET

✓QG3.0 is the best for the validation in cell (Kd value, dynamic range)

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

Quantification of GSH concentration in different cell lines

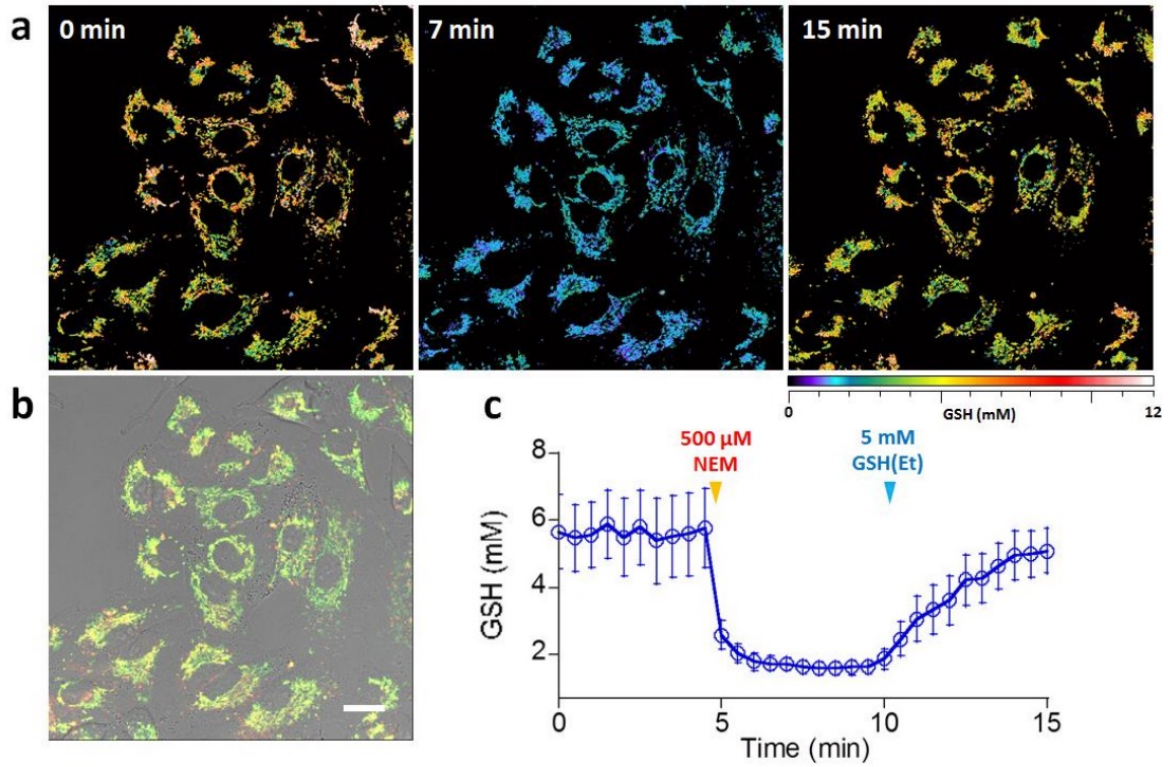




- ✓Different intracellular GSH concentrations were detected not only between different cell lines, but also between the same cell lines.
- ✓Each value was consistent with previously reported values.

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

Real-time monitoring of GSH changes induced by thiol scavenger



✓ QG3.0 can detect intracellular GSH in real time, in a reversible manner.

Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

Short summary

Main

✓Rationally designed and synthesized reversible fluorescent probes (QuicGSH) that enable real-time monitoring of GSH concentrations in living cells

✖ Accumulate to mitochondria due to the cationic specificity

（Mitochondrial GSH concentrations do not necessarily represent homogeneous intracellular GSH concentrations）

Problems in detecting localized intracellular GSH concentrations

Cells harbor several redox systems, which

- appear to be kinetically controlled
- not in equilibrium with each other
- have distinct regulatory functions
- are themselves subject to independent regulation.

roGFP (redox-sensitive GFP)

✓Measure the GSH/GSSG ratio

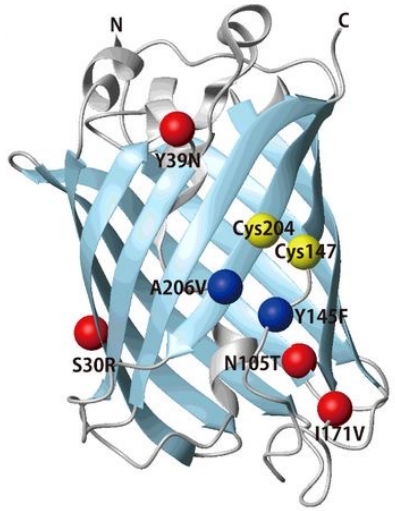
✓high response rate by fusing with Grx1

✓Localized observation is possible by expressing on specific organelles (like ER)

✖Operate at short wavelengths (<450 nm)

✖Display modest brightness

✖Limited dynamic ranges



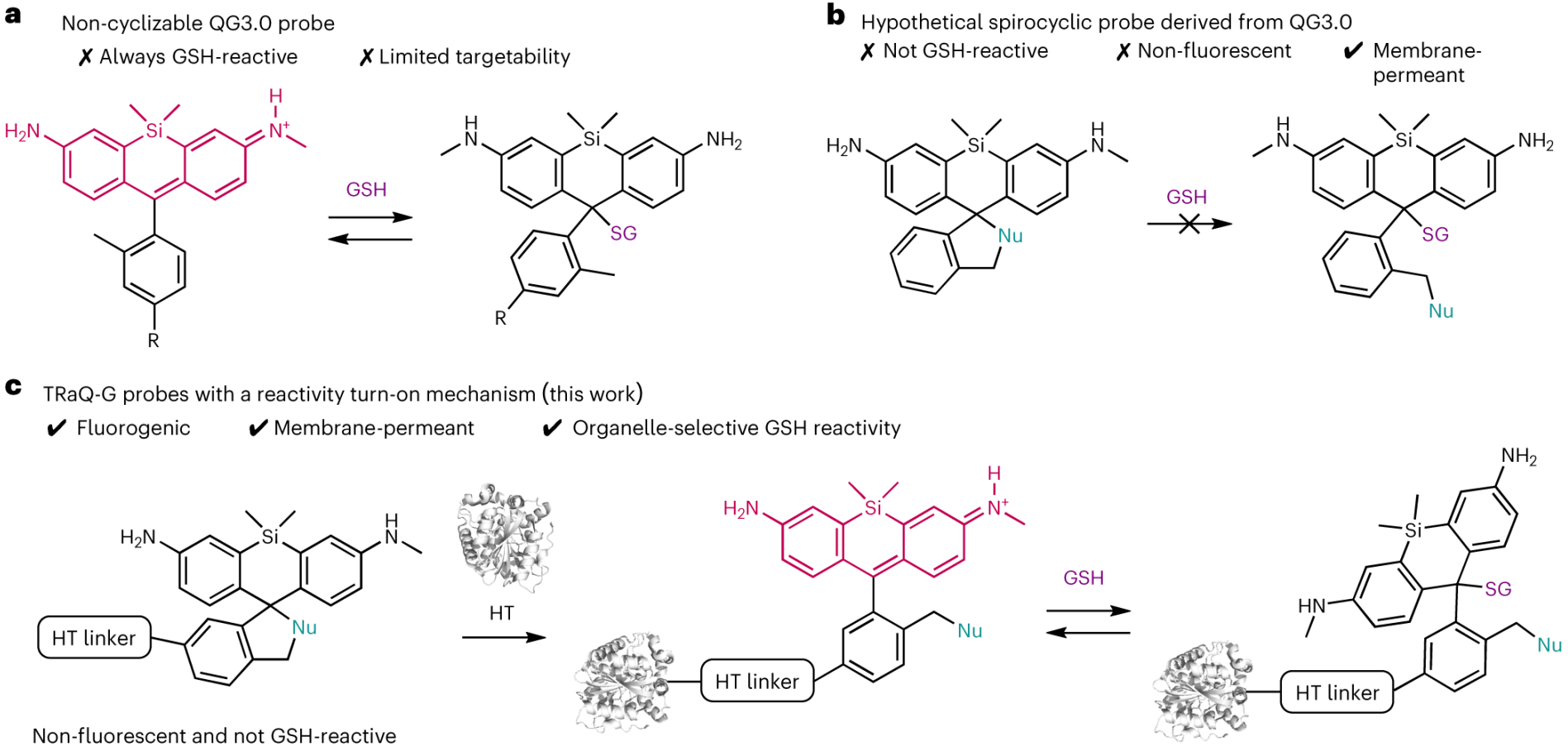
$$\mathrm{roGFP_{red}} + \mathrm{GSSG} \overset{K_{eq}}{\leftrightarrow} \mathrm{roGFP_{oxi}} + 2\mathrm{GSH}$$

$$K_{eq} = \frac{[\mathrm{roGFP_{oxi}}]\,[\mathrm{GSH}]^2}{[\mathrm{roGFP_{red}}]\,[\mathrm{GSSG}]}$$

Dick, T. et al, *Nat. Methods.* **2008**, 5, 553-559. Sakai, Y. et al, *Biosci. Rep.* **2016**, 36, e00316.

Previously reported SiR-based GSH sensors and the TRaQ-G sensing concept.

Main

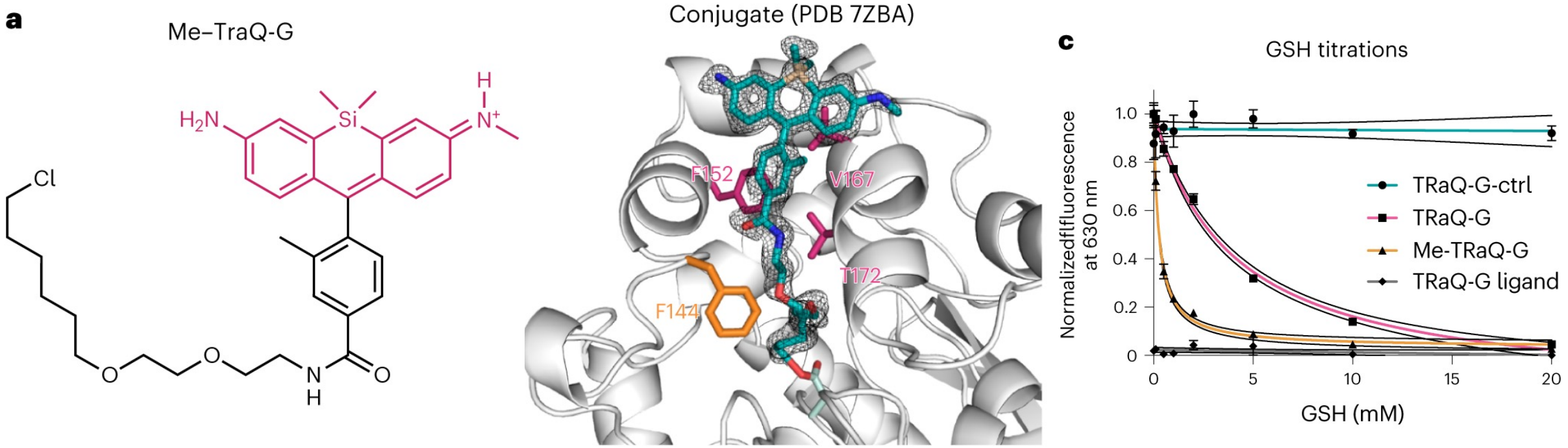


✓The use of HaloTag is expected to quantify local GSH concentrations in cells.

TRaQ-G : targetable ratiometric quantitative GSH probe

Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

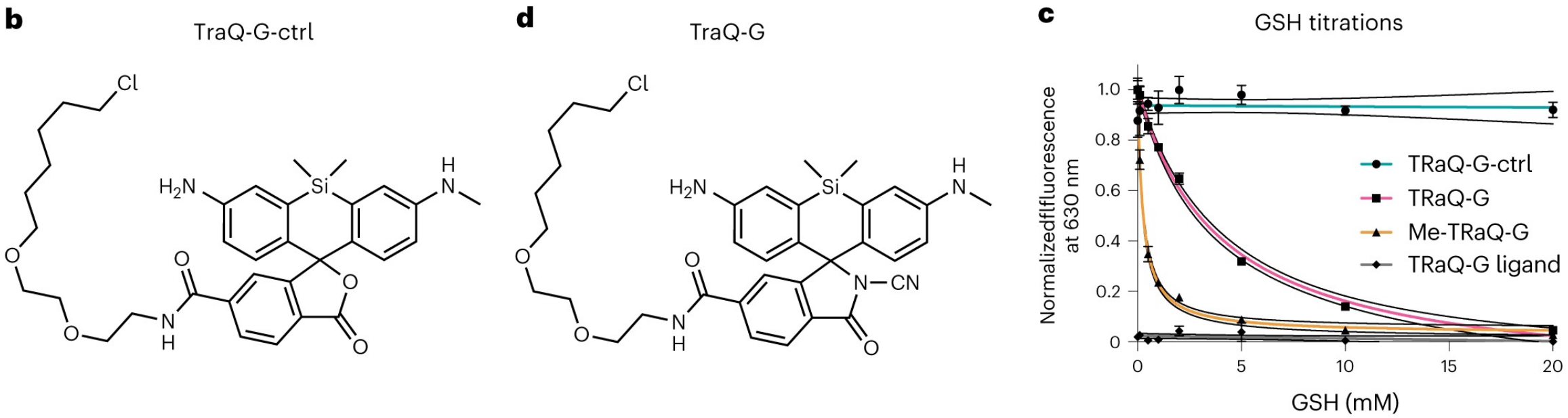
Structure–reactivity analysis of TRaQ-G probes



- ✓The presence of chloroalkane phosphor does not affect binding to GSH.
- ✓X-ray crystallographic analysis shows that the Halo Tag conjugate can adopt a conformation in which GSH is easily reacted with the Halo Tag.

Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

Structure–reactivity analysis of TRaQ-G probes

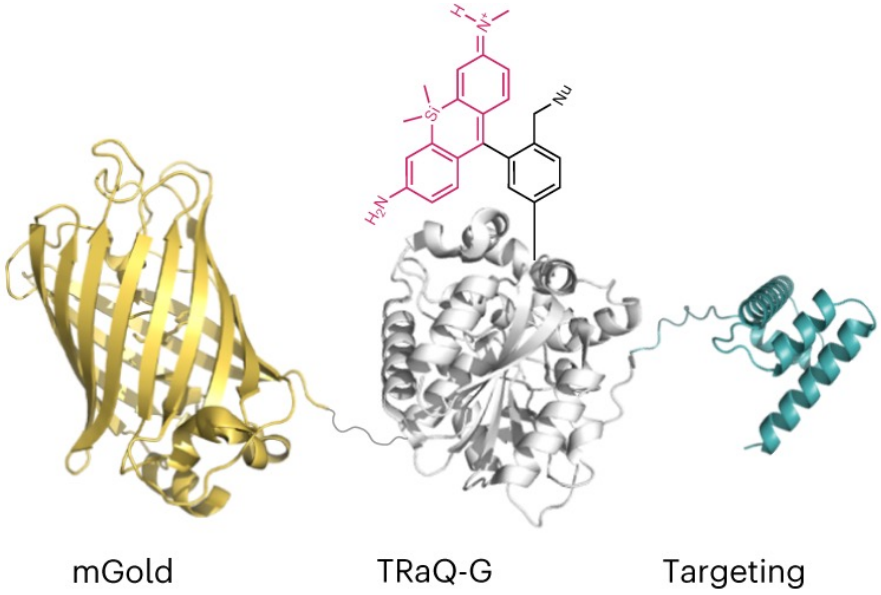


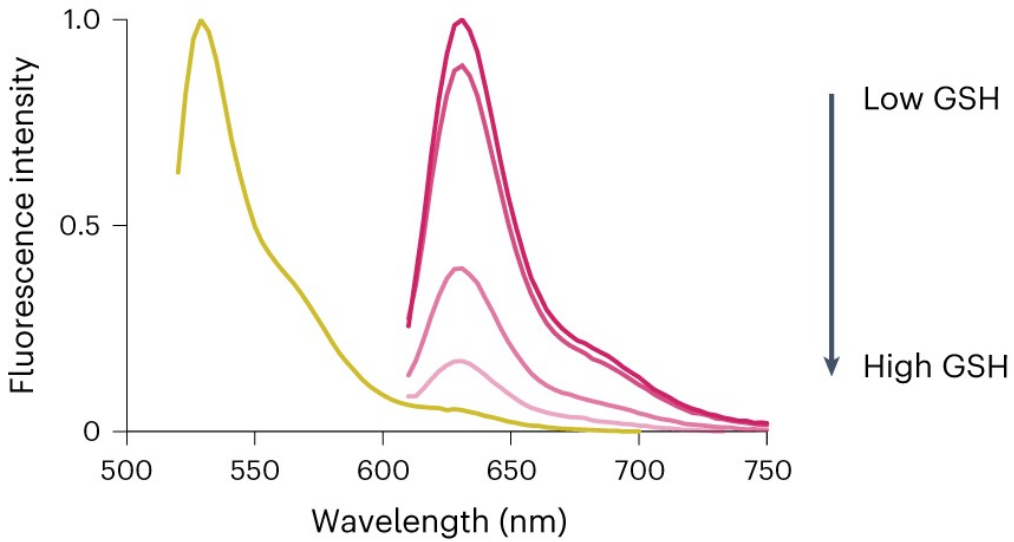
- ✓Carboxylic acid-derived spirocyclide are less reactive with GSH. (TraQ-G-ctrl)
- ✓Cyanamide-derived spirocyclide is highly reactive with GSH. (TraQ-G)

Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

TRaQ-G is a robust, targetable and quantitative GSH sensor

Main

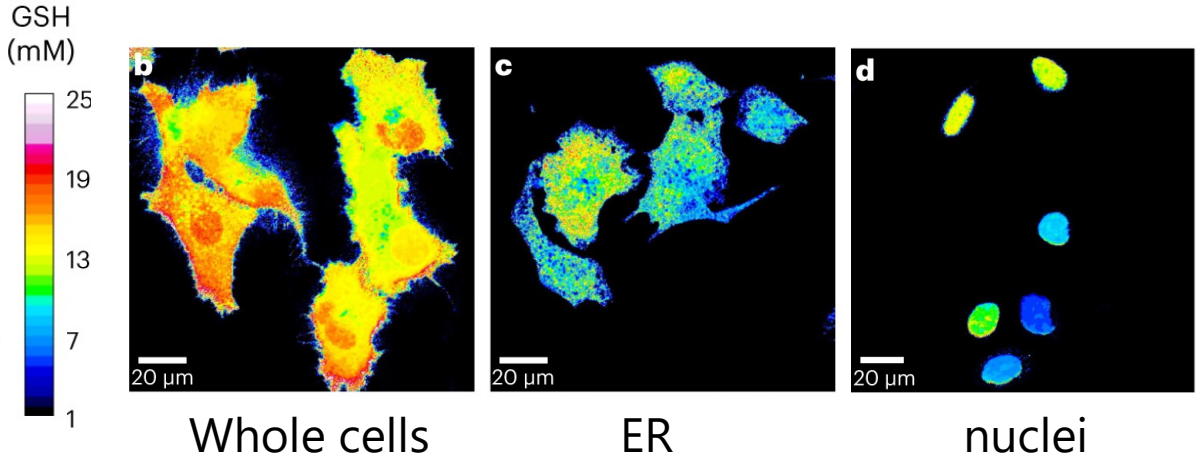




- ✓Using mGold as an internal standard, GSH can be quantified.
- ✓This construct could also be targeted to specific organelles using established peptide localization sequences.

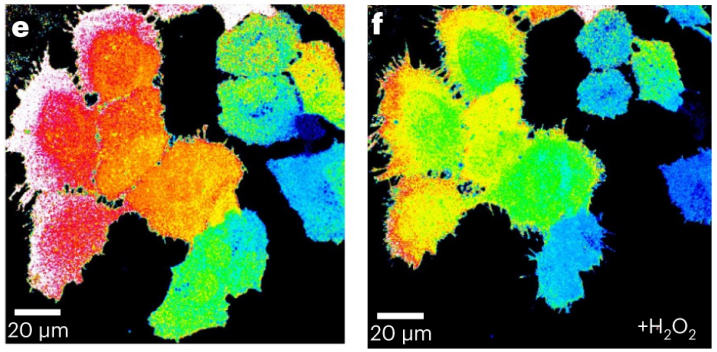
Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

TRaQ-G is a robust, targetable and quantitative GSH sensor

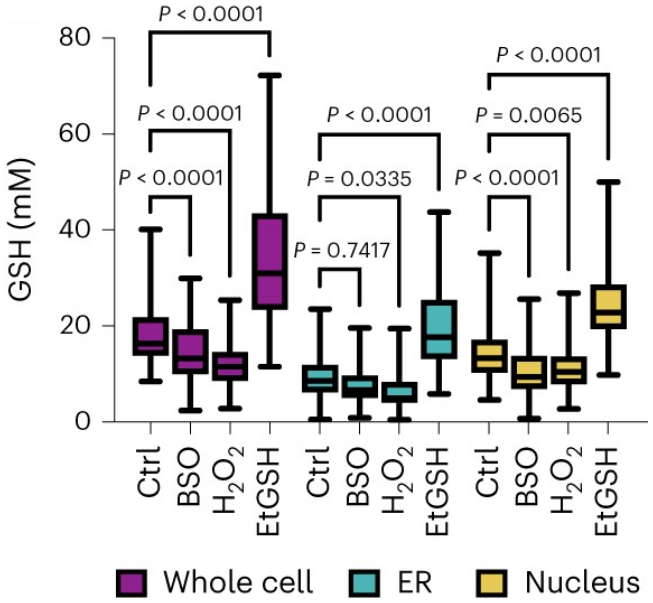


Whole cells ER nuclei

✓ Successful quantification of GSH at various intracellular targets.



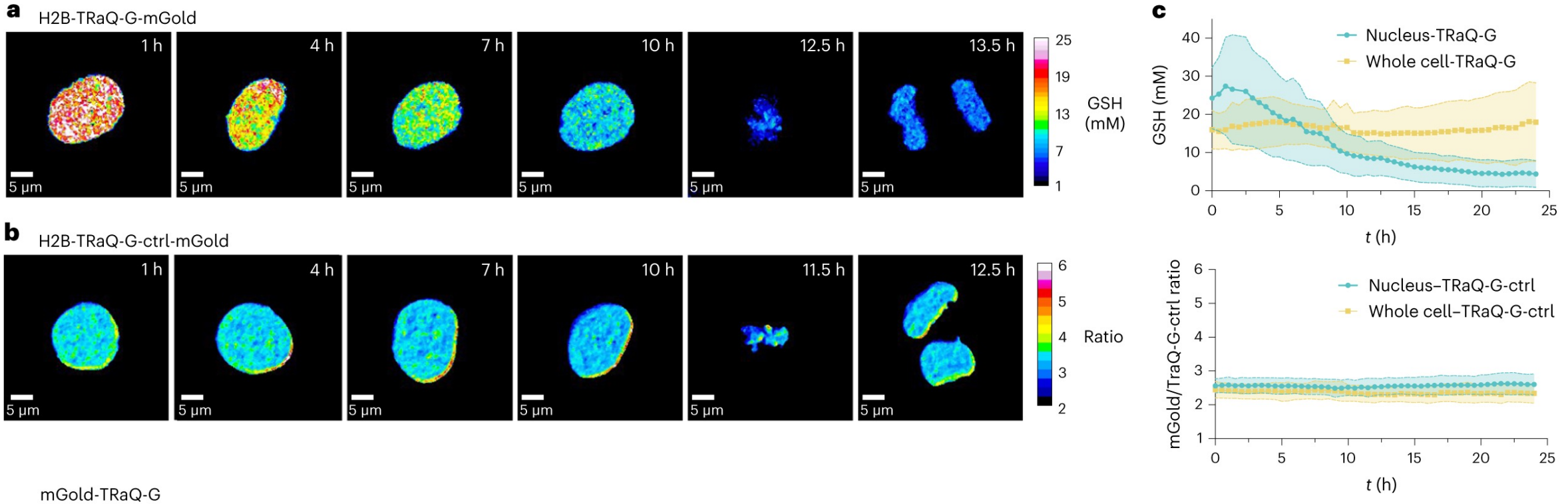
Whole cells Whole cells

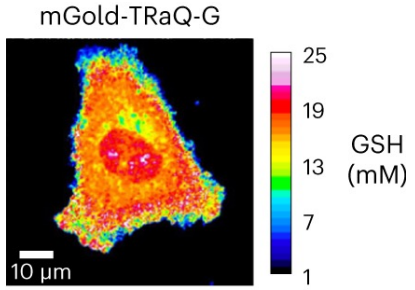


✓ Detect the changes in GSH concentration.

Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

Nuclear and cytosolic GSH pools are independently regulated

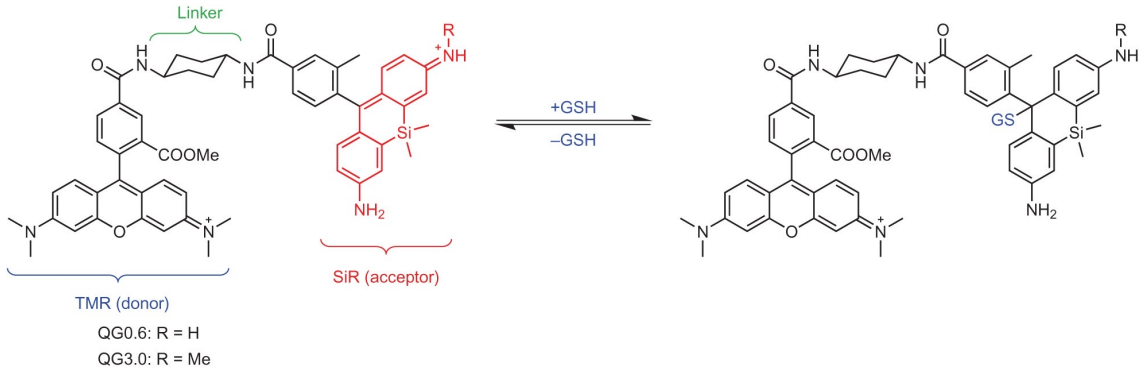


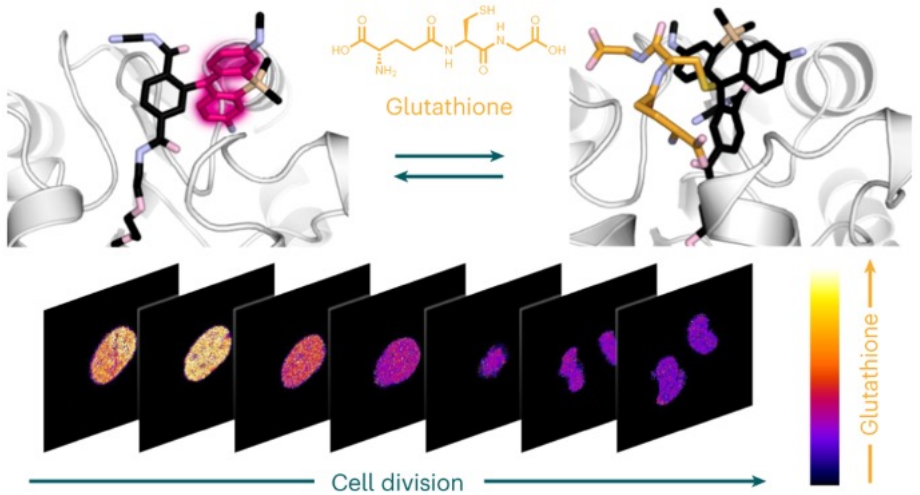


- ✓ Nuclear GSH concentrations were found to be regulated independently of other locations in the cell.
- ✓ GSH concentrations were shown to be highest in S phase and decreased steadily toward mitosis.

Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

- ✓A small molecule-based quantitative, live-imaging GSH probe has been developed.
- ✓The combination of tag proteins enables organelle-specific observation in cells.





Urano, Y. et al, *Nat. Chem.* **2017**, 9, 279-286.

Rivera-Fuentes, P. et al, *Nat. Chem.* **2023**, 1-7.

Thank you for your kind attention