Photochromic Fluorescent Probe

Literature Seminar

2018/7/21

M2 Takehiro Kato

Contents

1. Introduction
2. Intracellular target recognition by photochromic fluorescent probe (main paper)

ARTICLE

DOI: 10.1038/s41467-017-01137-8 OPEN

Remote light-controlled intracellular target recognition by photochromic fluorescent glycoprobes

Junji Zhang[1], Youxin Fu[1], Hai-Hao Han[1,2], Yi Zang[2], Jia Li[2], Xiao-Peng He[1], Ben L. Feringa[3] & He Tian[1]

3. “Double-check” bioimaging enabled by probe-protein hybrid

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Communication
Cite This: J. Am. Chem. Soc. 2018, 140, 8671−8674
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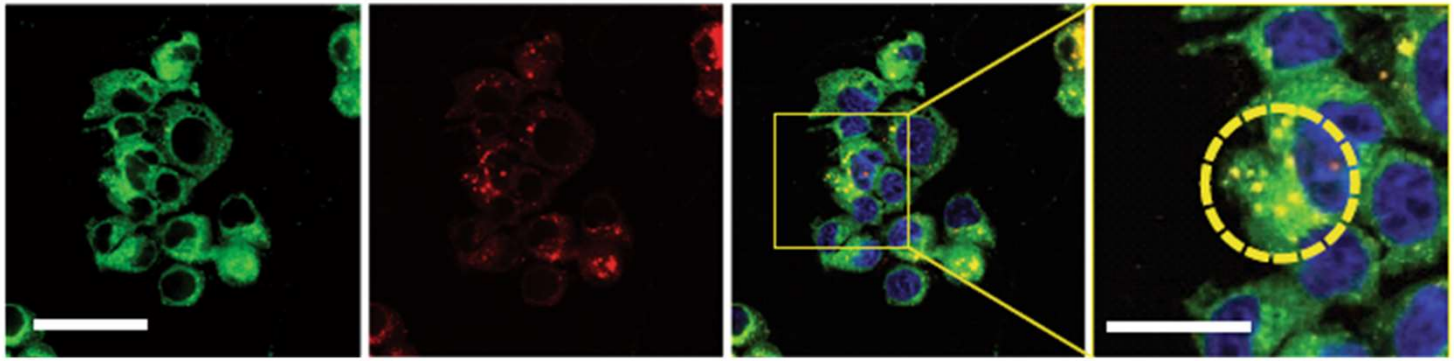
Photocontrolled Fluorescence “Double-Check” Bioimaging Enabled by a Glycoprobe−Protein Hybrid

Youxin Fu,[†,§] Hai-Hao Han,[†,§] Junji Zhang,[*,†] Xiao-Peng He,[*,†] Ben L. Feringa,[†,‡] and He Tian[*,†]

Fluorescent Imaging



Intracellular imaging of biomolecules with fluorescent probe

- **noninvasive**
- **precise and rapid detection**
- **spatial and temporal accuracy**

But...

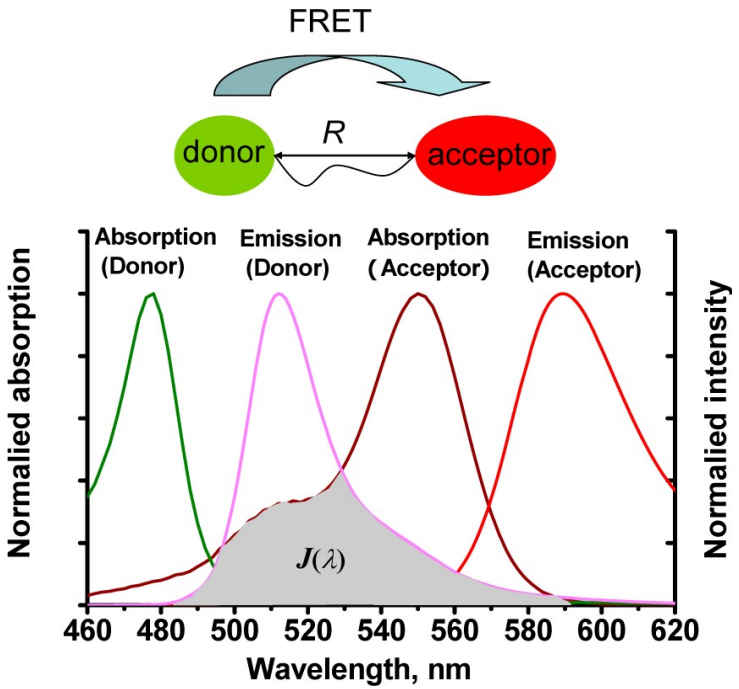
- **interfered by microenvironment (pH, salt strength, biomacromolecules, ...)**
- **biological autofluorescence**

-> Fluorescence probes which does not rely only on single emission intensity is needed.[1)]

- **ICT (Internal Charge Transfer)**
- **FRET (Förster Resonance Energy Transfer), TBET (Through Bond Energy Transfer)**
- **ESIPT (Excited-State Intramolecular Proton Transfer)**
- **Monomer-excimer**

1) Lee, M. H.; Kim, J., S.; Sessler, J. L. *Chem. Soc. Rev.*; **2015**, *44*, 4185.

FRET (Förster Resonance Energy Transfer)

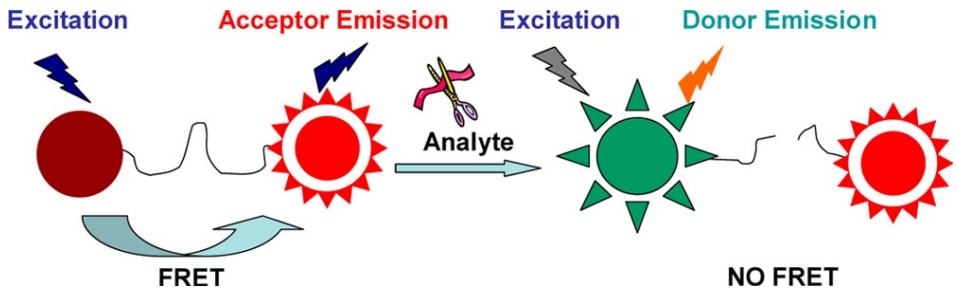


FRET: nonradiative energy transfer from donor to acceptor through long-range dipole-dipole interactions

FRET efficiency: $E = R_0^6/(R_0^6+R^6)$

R_0 = Förster distance (constant)

R = distance between donor and acceptor

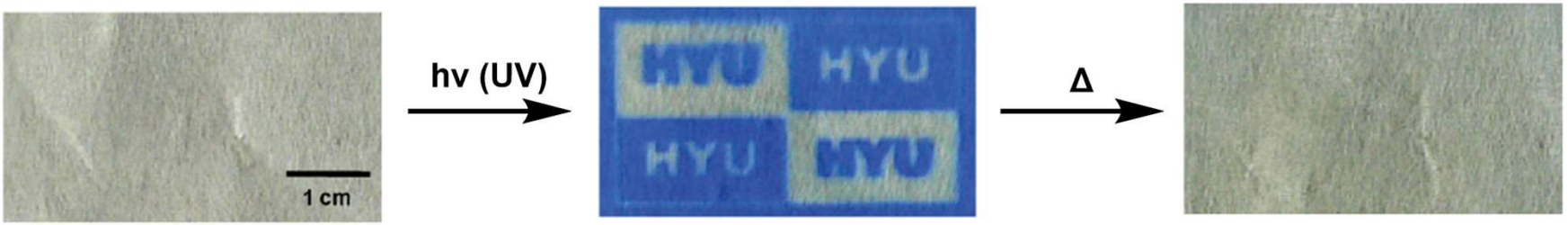


FRET switching is also induced by:

- **interaction between donor/acceptor and analyte**
- **removal of "capping" moiety**
- **photochromic structural conversion (today's topic)**

Yuan, L.; Lin, W.; Zheng, K.; Zhu, S. *Acc. Chem. Res.*, **2013**, *46*, 1462.

Photochromism



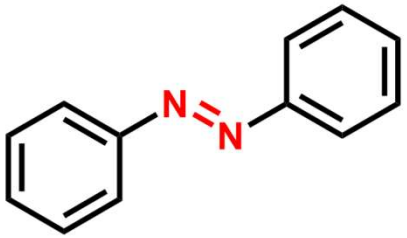
*Source of figures: ref. 1

Definition of photochromism[2]:

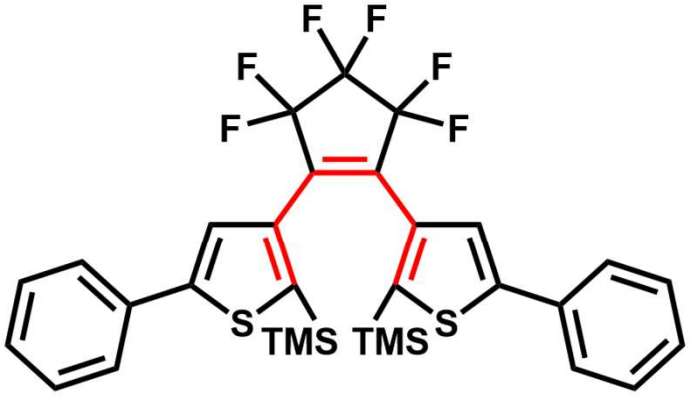
light-induced reversible transformation between two isomers having different absorption spectra

Examples:

azobenzene (*cis-trans* isomerization)
dithienylethene (6π electrocyclic reaction)
spiropyrans (-> today's topic)



azobenzene



dithienylethene

1) Lee, J.; Lee, C.-W.; Kim, J.-M. *Macromol. Rapid Commun.*, **2010**, *31*, 1010. 2) Kobatake, S.; Irie, M. *Annu. Rep. Prog. Chem., Sect. C*, **2003**, *99*, 277.

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Photocontrolled Fluorescence "Double-Check" Bioimaging Enabled by a Glycoprobe−Protein Hybrid

Youxin Fu,[†,§] Hai-Hao Han,[†,§] Junji Zhang,[*,†] Xiao-Peng He,[*,†] Ben L. Feringa,[†,‡] and He Tian[*,†]

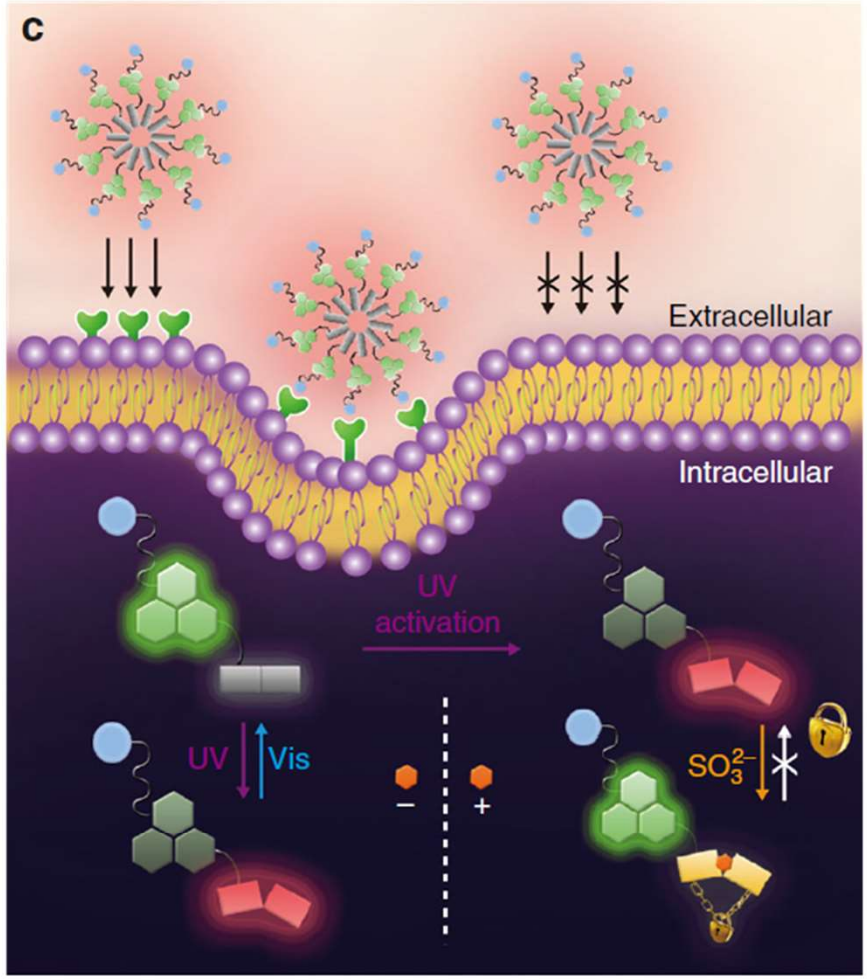
Prof. He Tian

1982 B.S. Nanjing University of Science and Technology
1986 M. Sc. East China University of Science and Technology (ECUST)
"Studies of Correlations between Photovoltaic Effect and Structures of Organic Dyes in Solar Cells"
1989 Ph.D. ECUST (Prof. Zhenghua Zhu)
"Studies on Spectral Sensitization and Supersensitization by Dember Effect"
1991-1993 Postdoc, University of Siegen, Germany
(Prof. Karl H. Drexhage)
"Intramolecular Energy and Electron Transfer in Multi-chromophoric Laser Dyes"
1994-present Full Professor, ECUST

Current research:
Functional organic dyes
Fluorescent chemosensors
Supramolecular machines
Organic photochromic materials

Concept of This Research

Aim 1.
Reversible shift between two isomeric states with remote control by light.

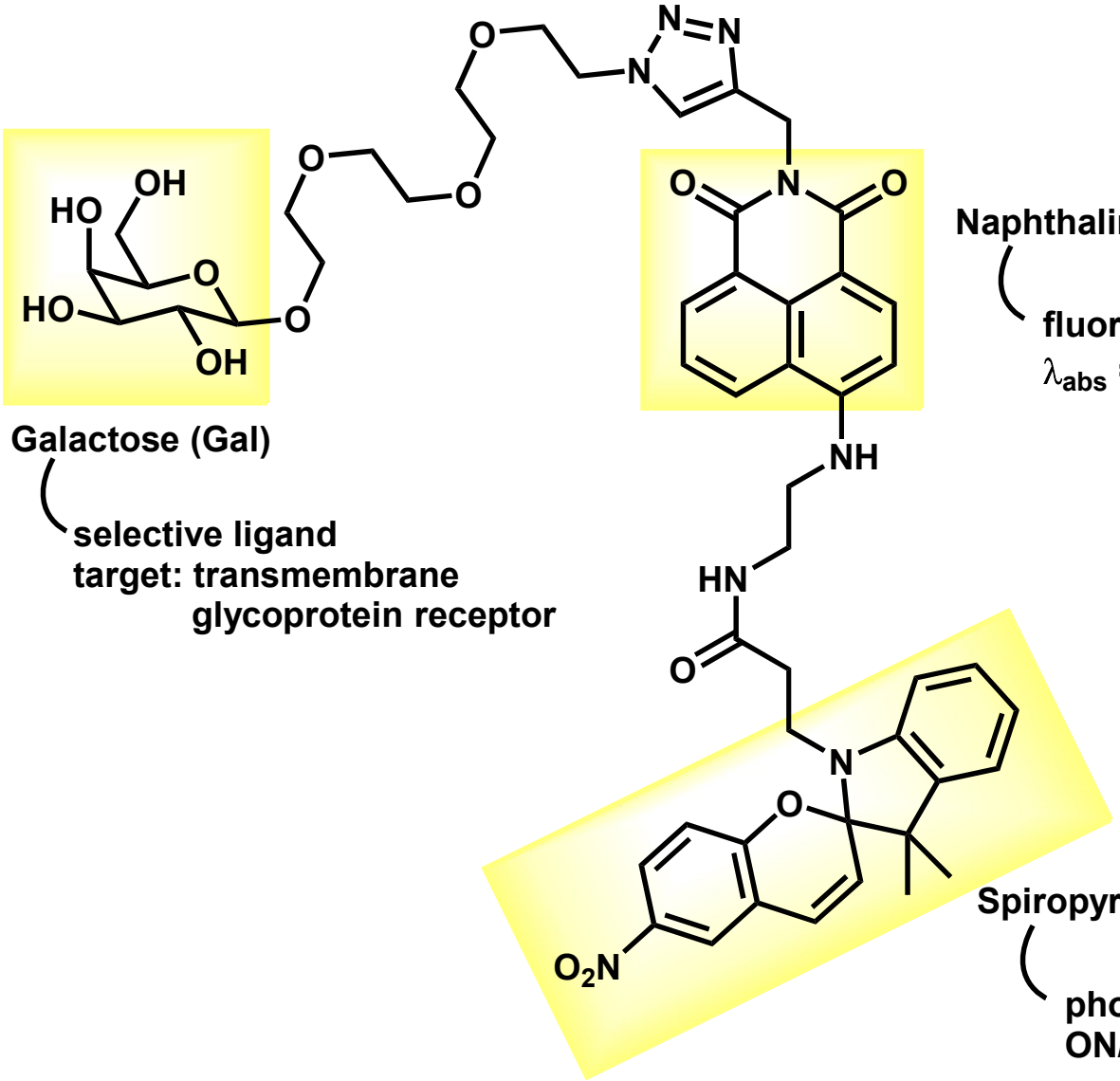


Aim 3.
Selective assembly in live cells with specific reseptors.

Aim 2.
Expression of probe reactivity just before analyte binding.

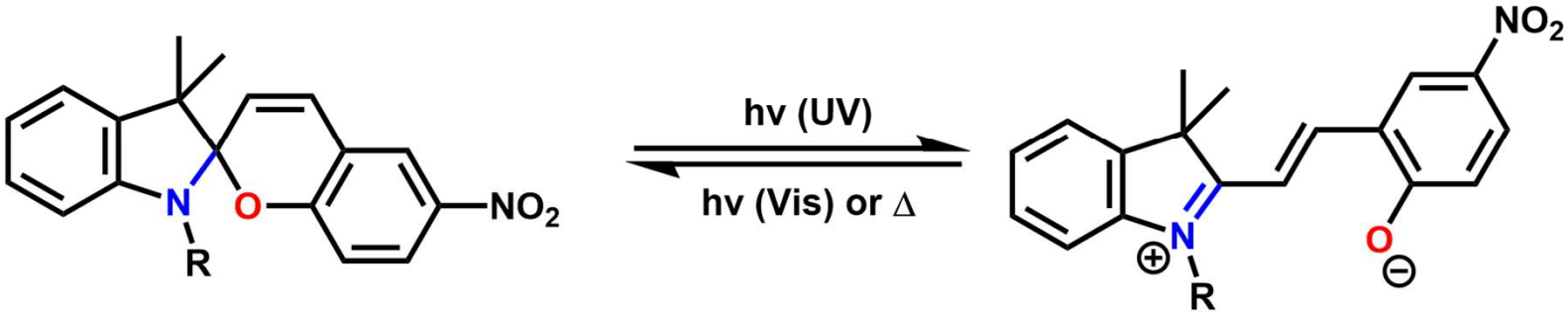
Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Molecular Design of SP-Gal



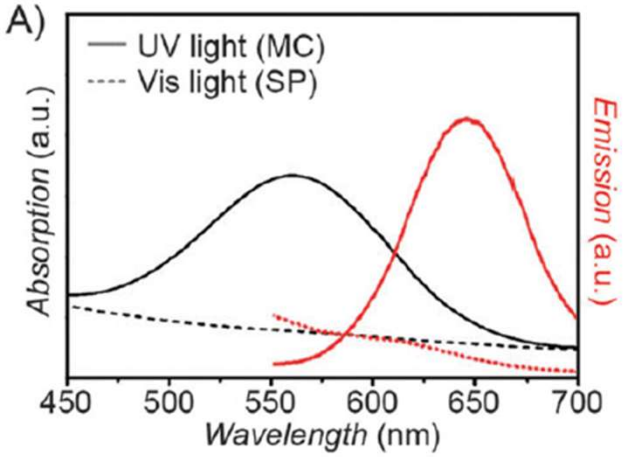
Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Spiropyran (SP) - Merocyanine (MC)



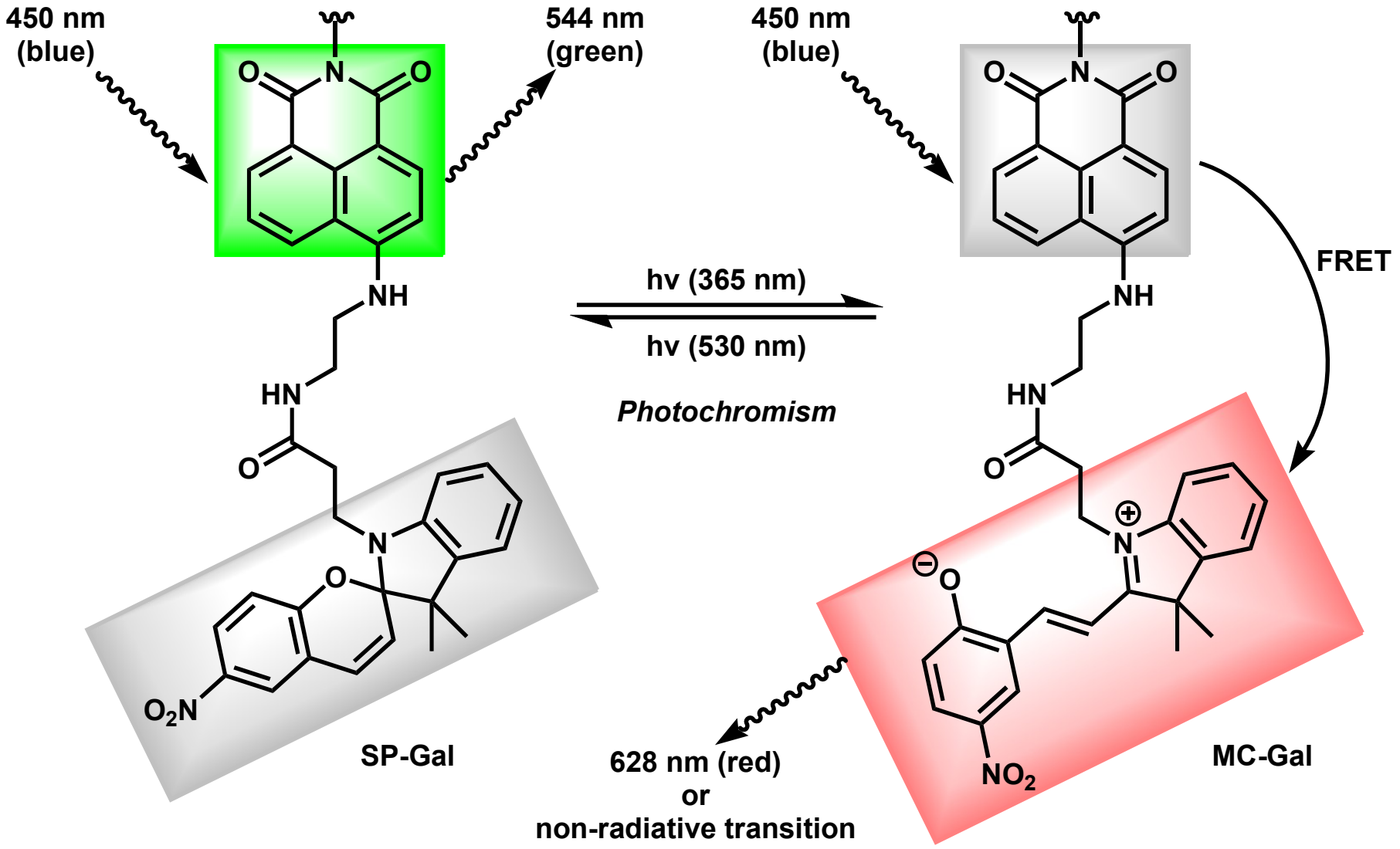
spiropyrane (SP)
- two conjugate systems
- neutral species
- λ_{abs} ~ 280 and 340 nm, no emission

Merocyanine (MC)
- one large conjugate system
- zwitter ionic species
- λ_{abs} ~ 560 nm, λ_{em} ~ 650 nm



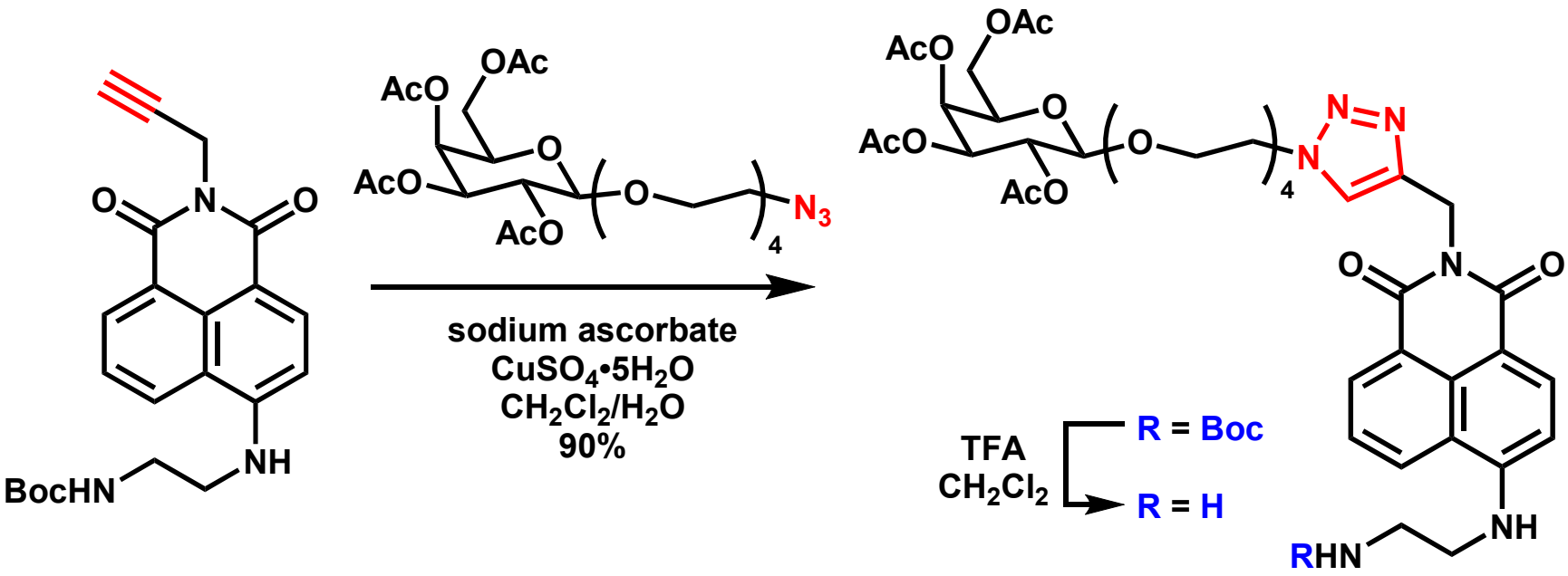
Klajn, R. *Chem. Soc. Rev.*, **2014**, *43*, 148.

Molecular Design for FRET-Photochromism



Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Synthesis of SP-Gal



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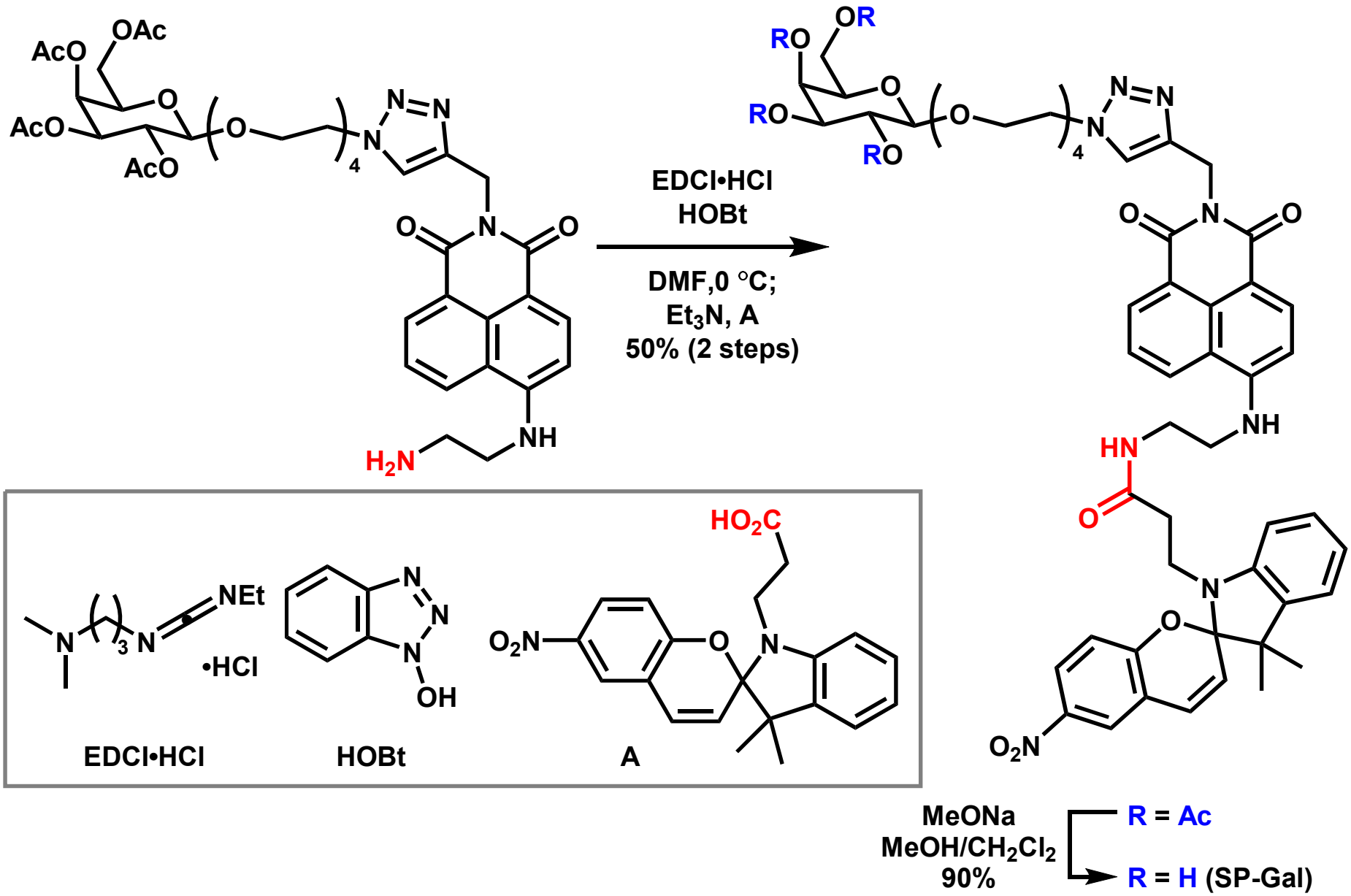
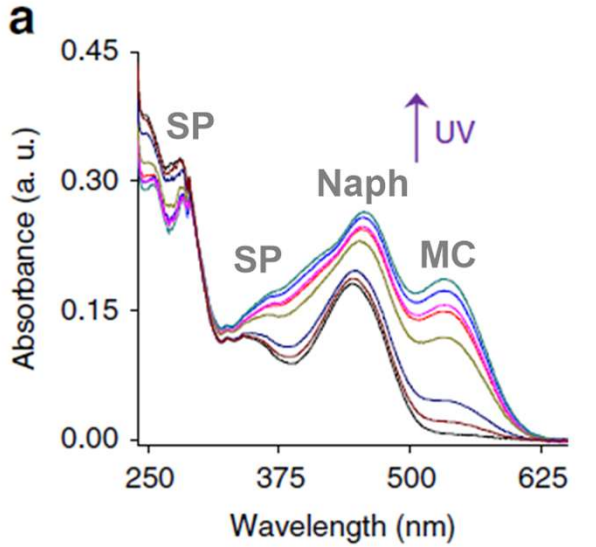
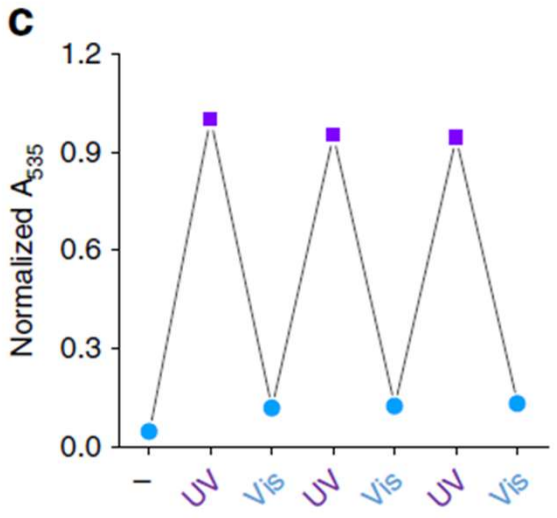
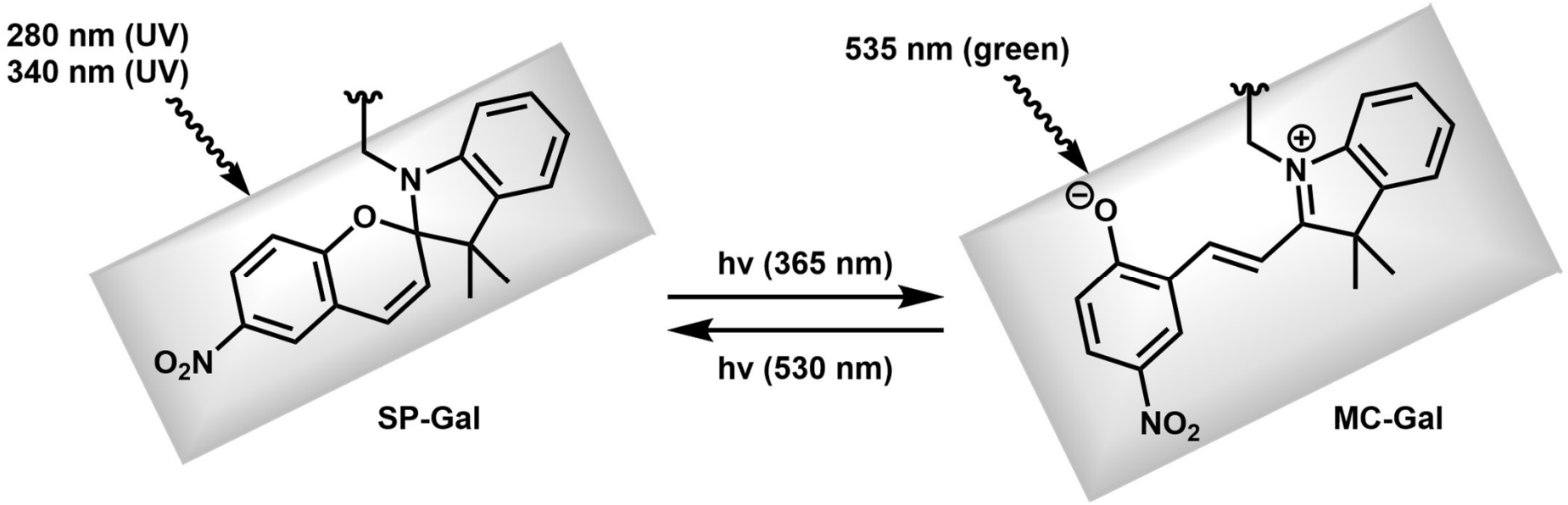


Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Absorbance Spectra of SP/MC-Gal

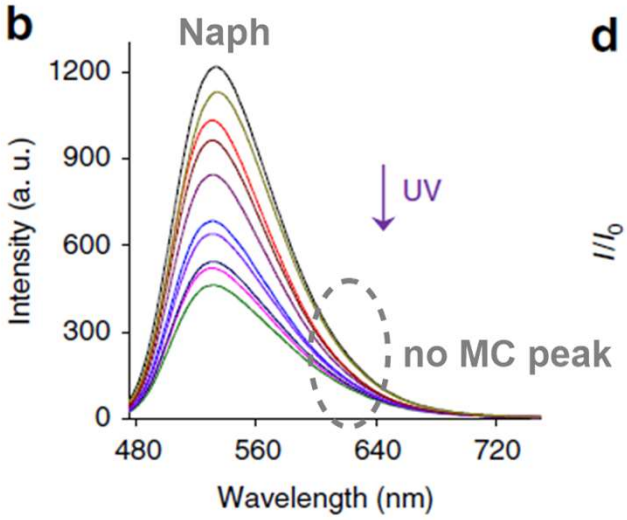


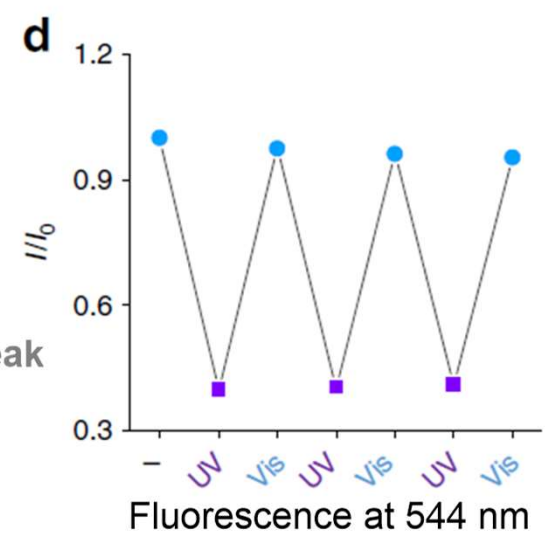


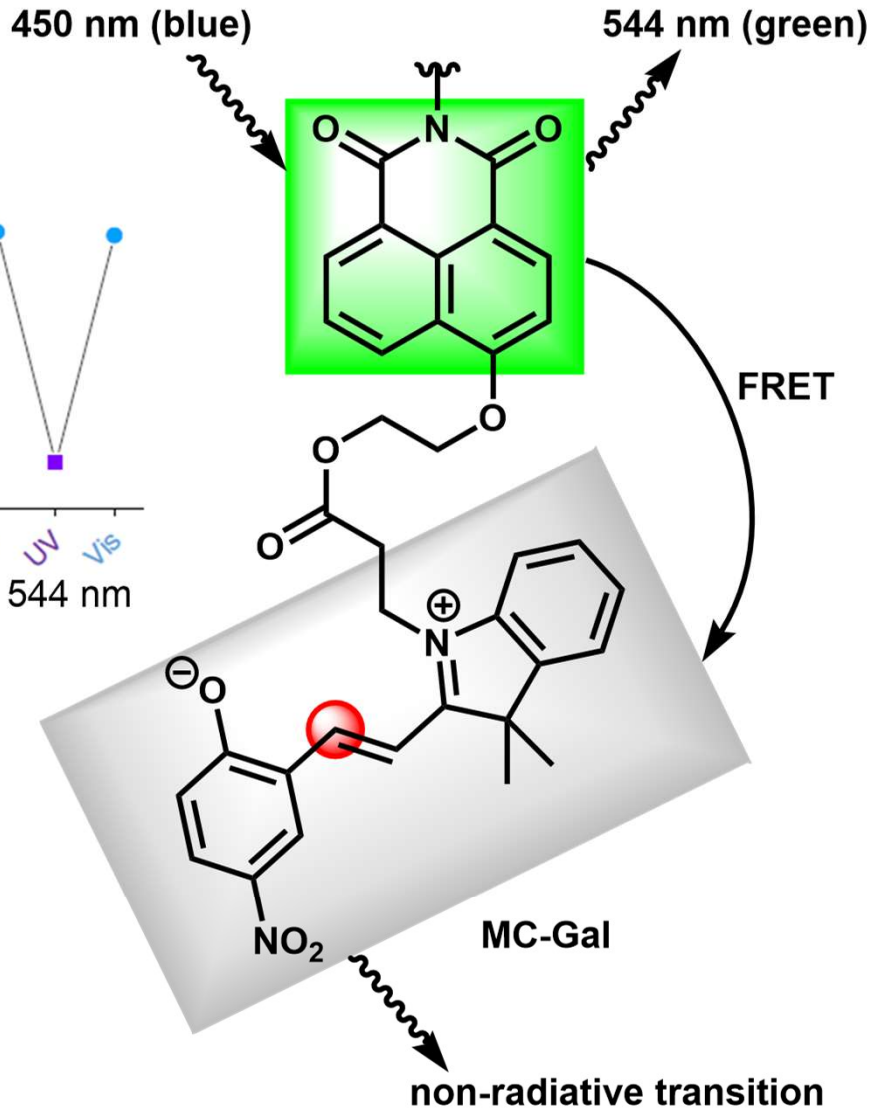


Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Fluorescence Spectra of SP/MC-Gal







- reversible transformation
- no apparent photo-degradation
- FRET by MC (FRET efficiency: 65.9%)
- non-radiative relaxation of MC

- electrophilicity of MC moiety?

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Sulfur Dioxide (SO_2)

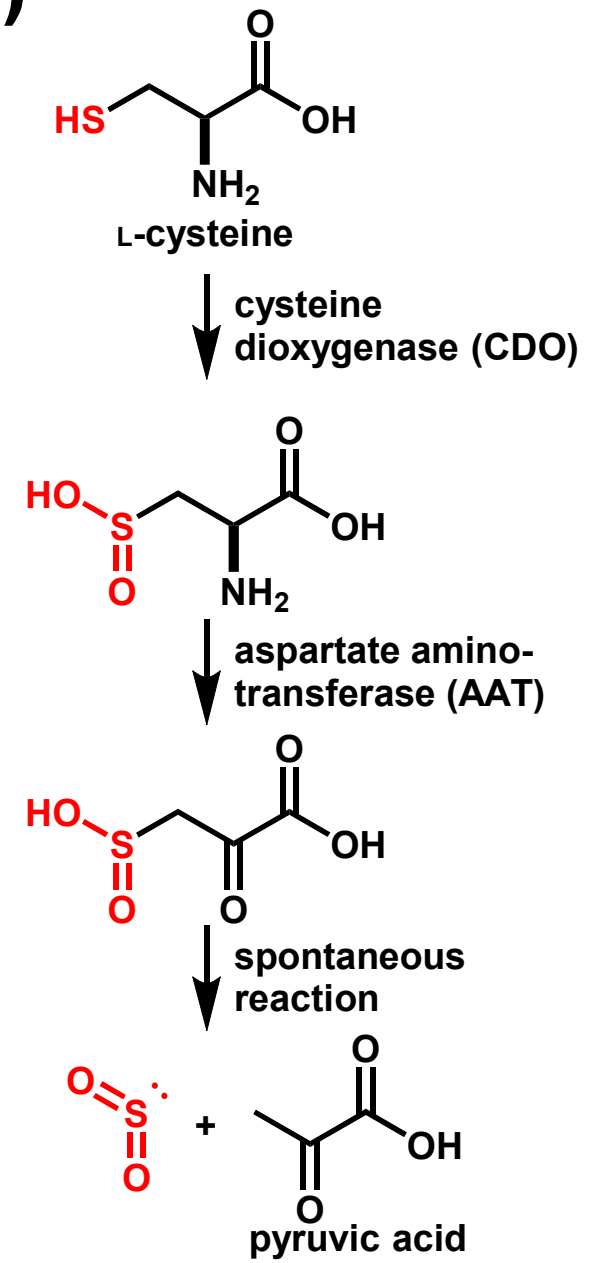
- environmental pollutant
  asthma, neurological disorders, cardiovascular diseases, ...

- exist in aqueous media as sulfite derivatives, HSO_3^- and SO_3^{2-}

- high nucleophilicity on sulfur atom
  (aldehyde -> α-hydroxy sulfonic acid)

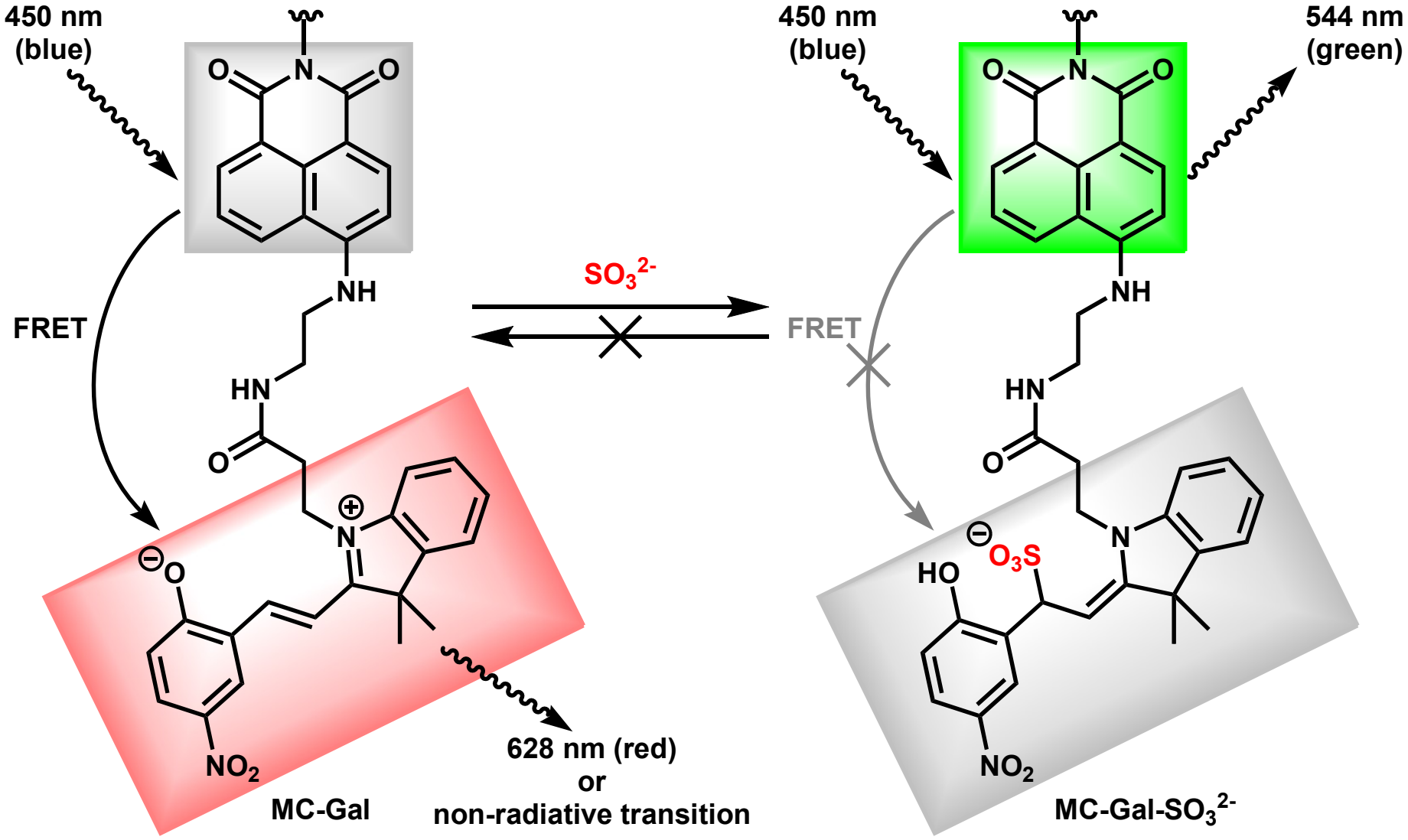
- endogenously generated from L-cysteine (see scheme)

- biological effect
  biomarker of acute pneumonia, renal failure
  important mediator in the cardiovascular system?
  ... but not fully understood in mammals.



Luo, L.; Chen, S.; Jin, H.; Tang, C.; Du, J. *Biochem. Biophys. Res. Commun.* **2011**, *415*, 61.

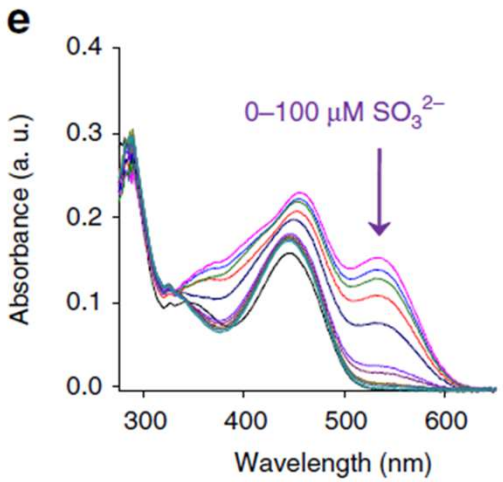
FRET-Inhibition by Sulfite Anion

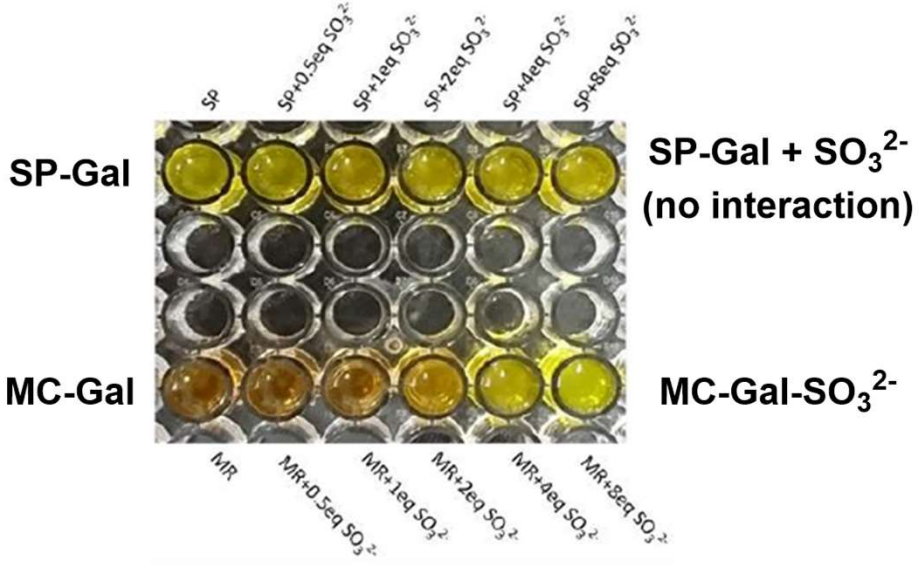


Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

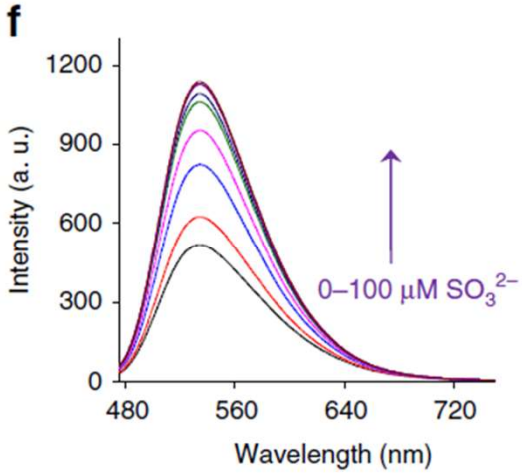
Spectra of MC-Gal with SO_3^{2-}

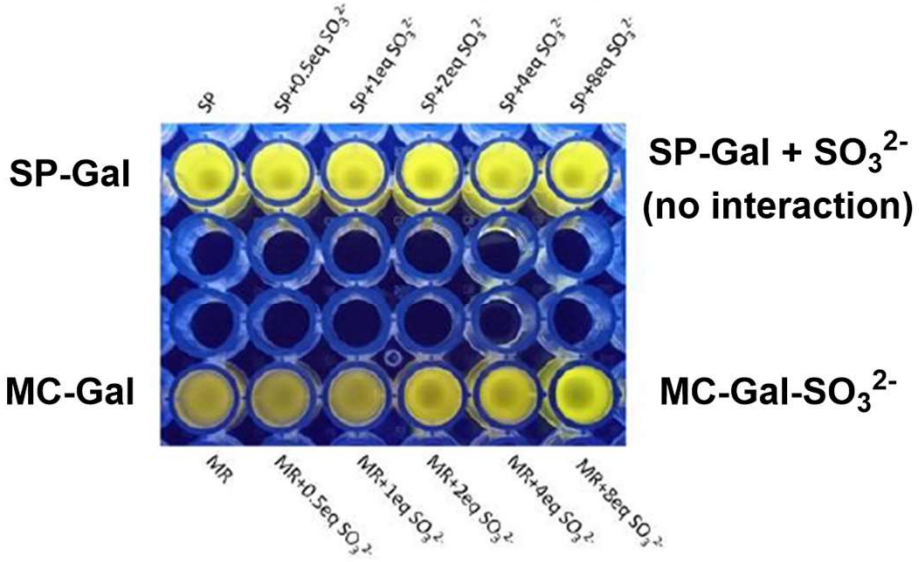
Absorbance





Fluorescence





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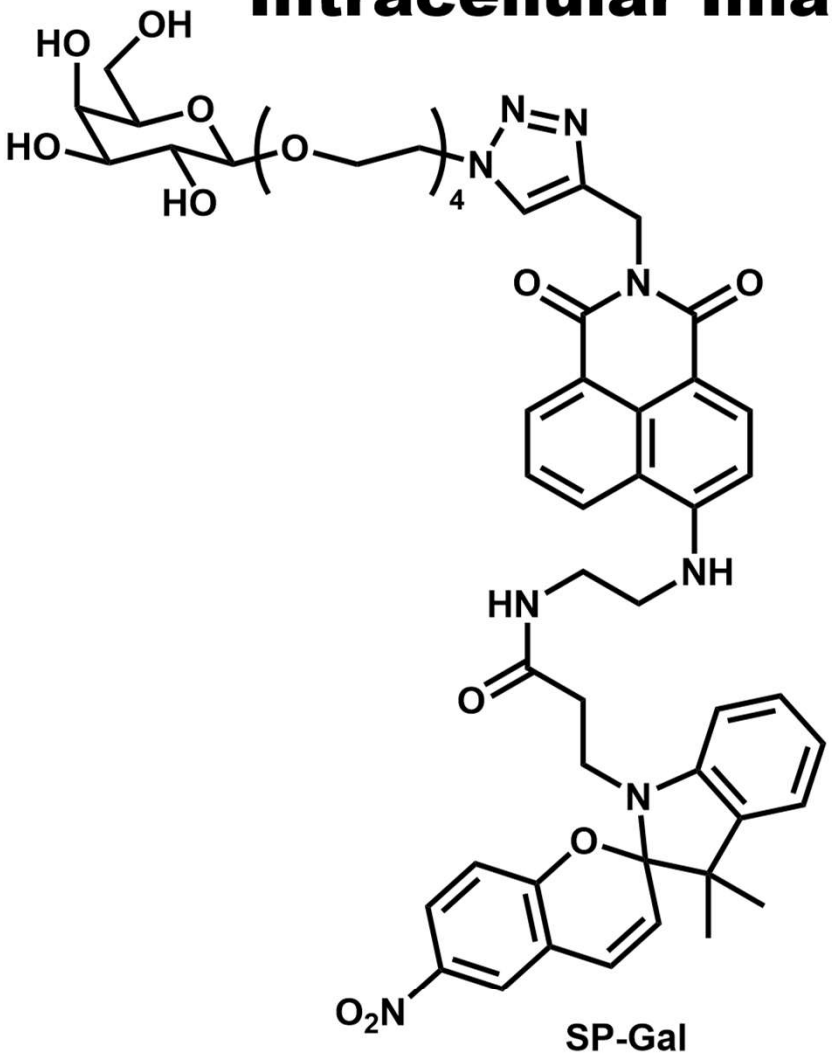
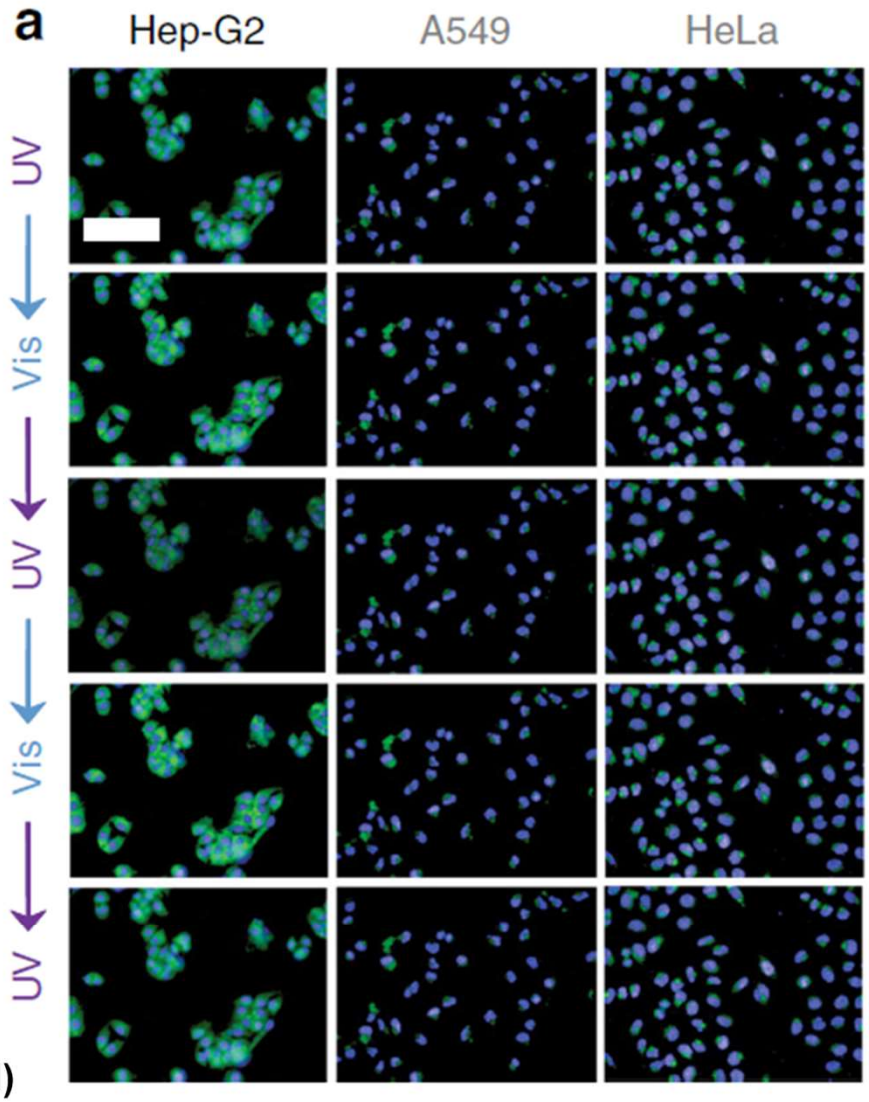
Intracellular Imaging by SP/MC-Gal



SP-Gal



Hep-G2: human hepatoma cell line
(asialoglycoprotein receptor, selective for Gal)
A549: human lung cancer cell line (control)
HeLa: human cervical cancer cell line (control)

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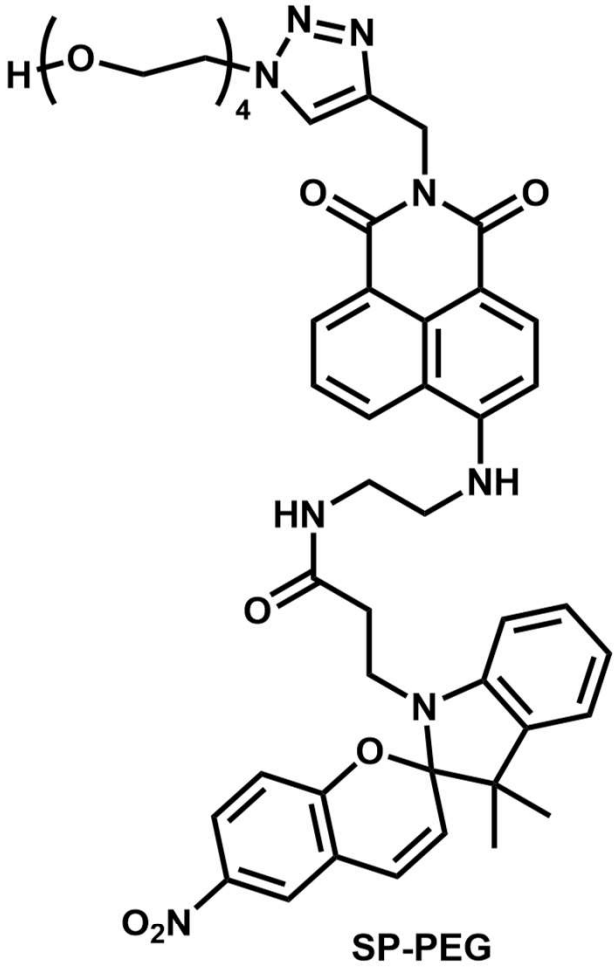
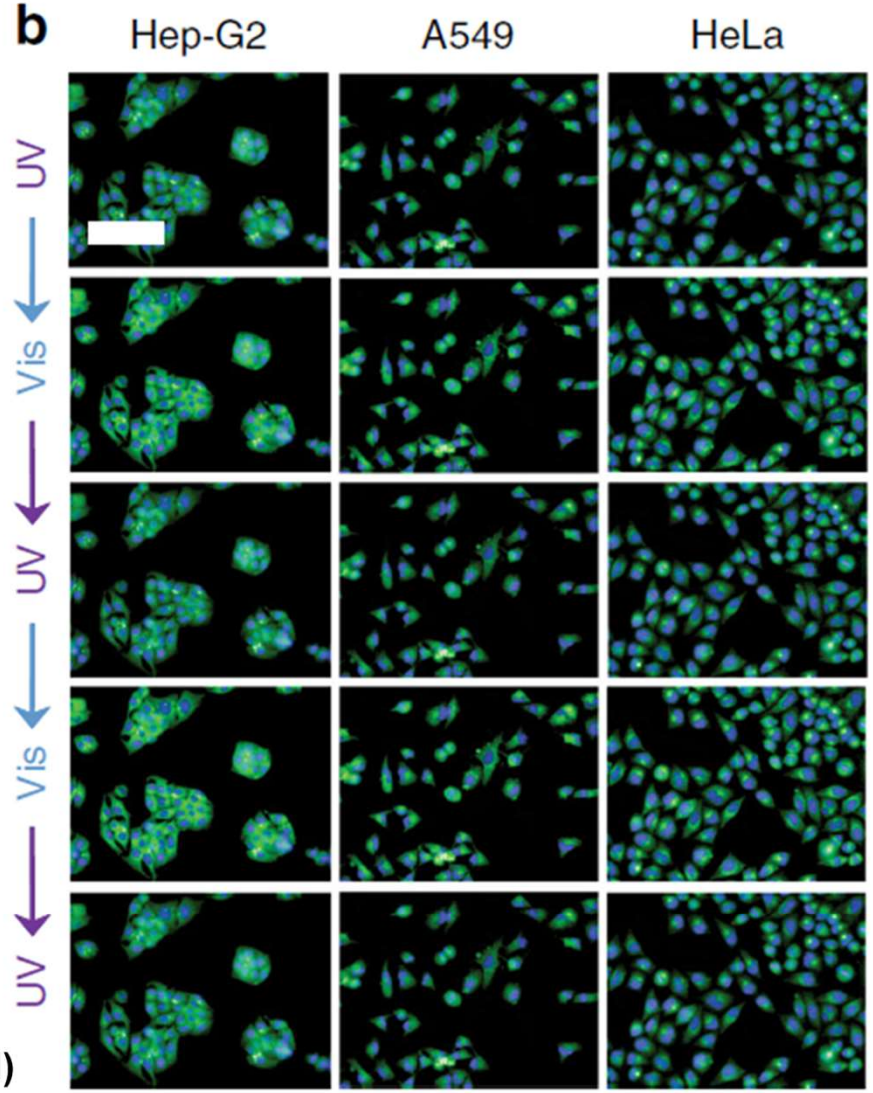
Intracellular Imaging by SP/MC-PEG



SP-PEG



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Co-localization with Lyso-Tracker

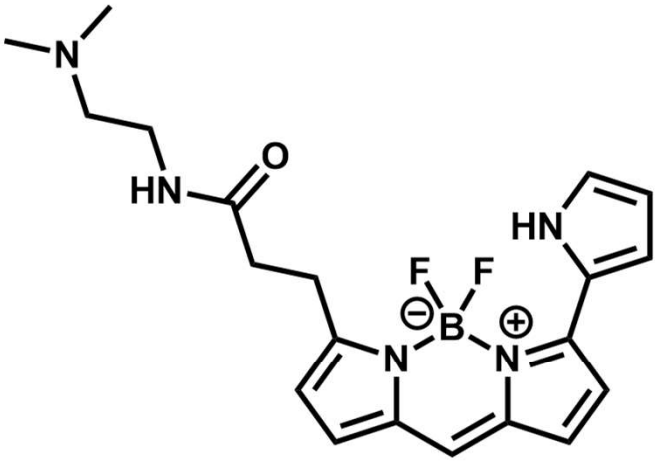
recognition by Gal receptor

↓

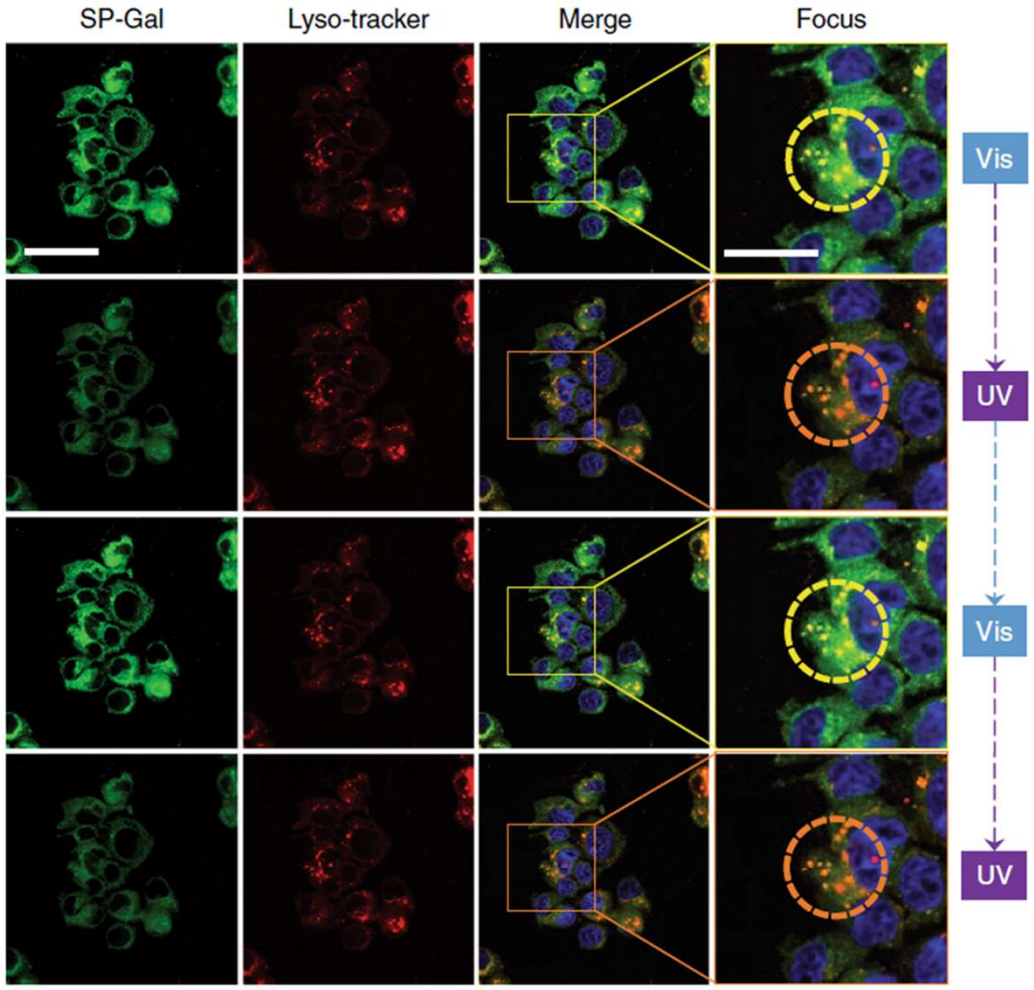
endocytosis

↓

transportation to lysosome



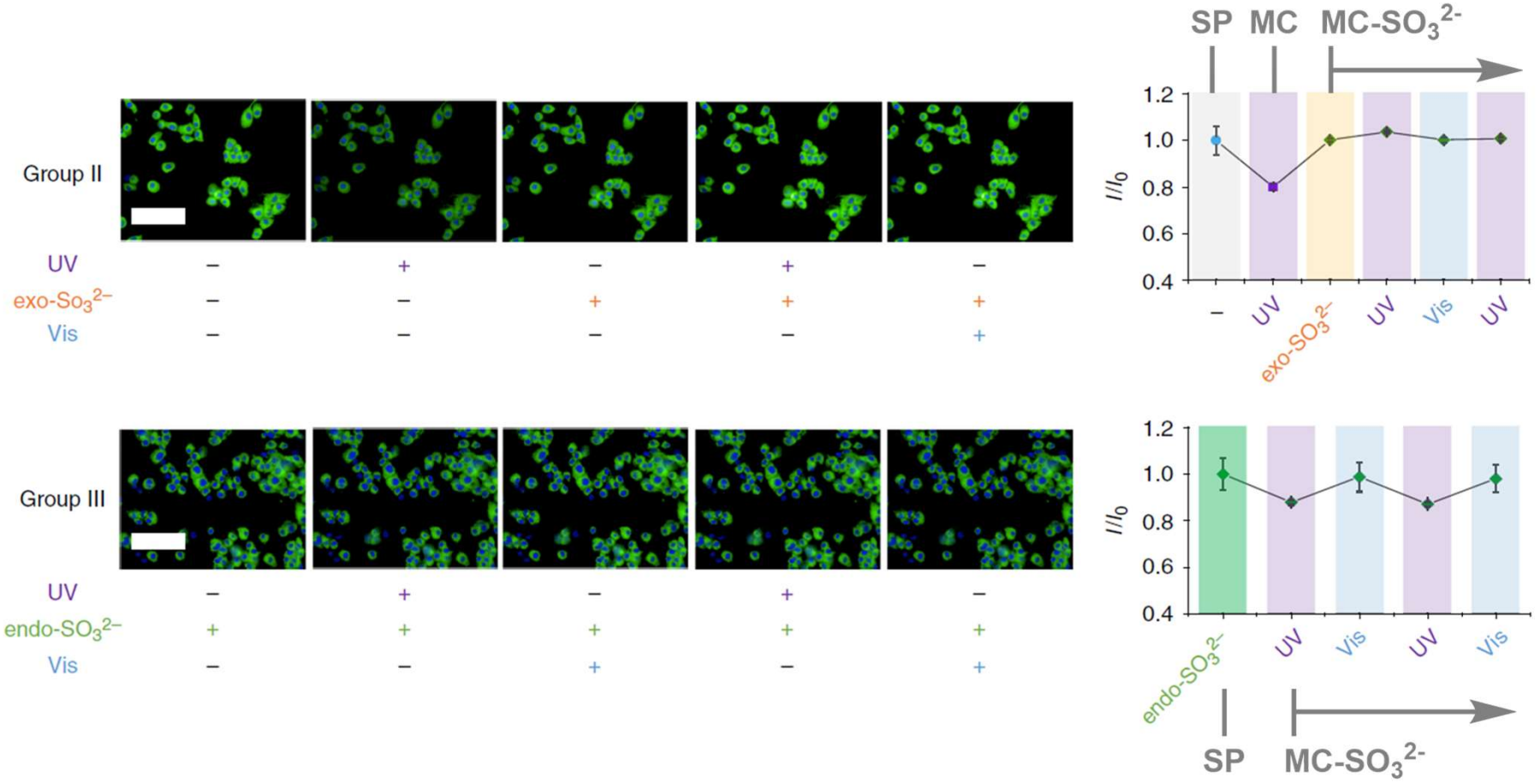
Lyso-Tracker Red



Pearson's correlation coefficient: 0.76

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Detection of Intracellular SO_3^{2-} by SP/MC-Gal[1)]



exogenous SO_3^{2-}: treatment of cells with Na_2SO_3

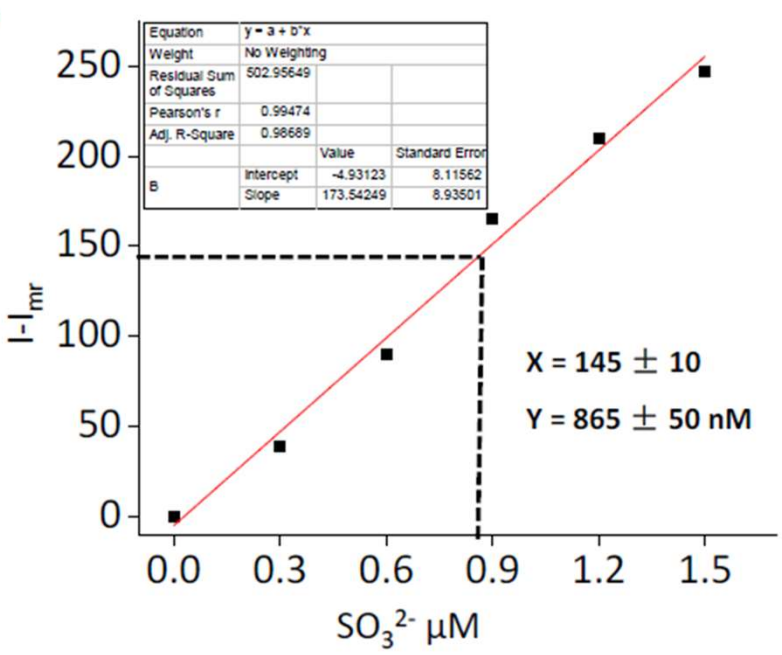
endogenous SO_3^{2-}: pretreatment of the cells with lipopolysaccharide[2)]

(induction of low-level SO_3^{2-})

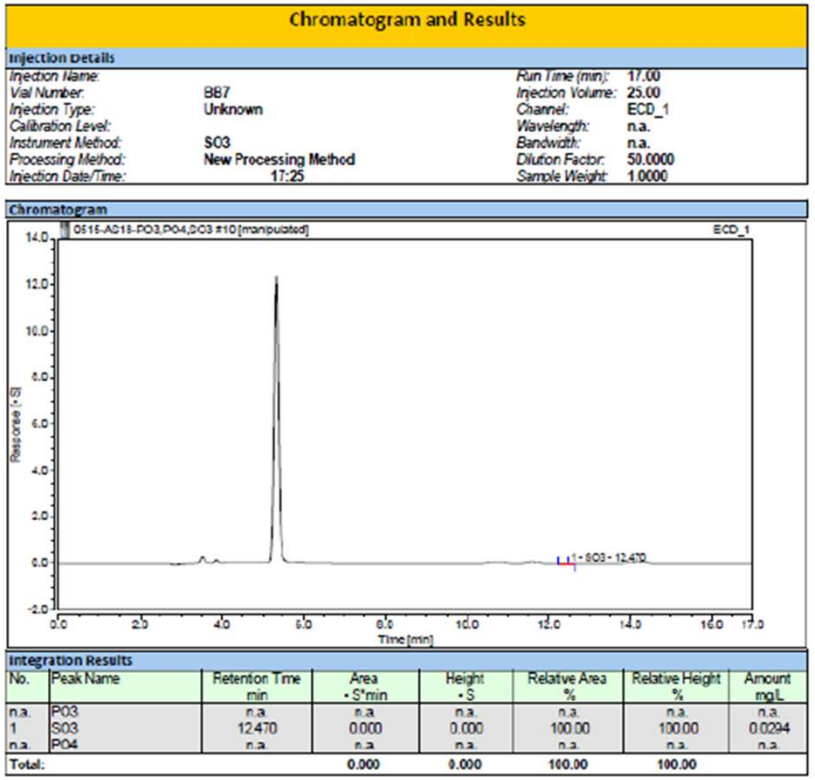
1) Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.
2) Mitsuhashi, H.; Nojima, Y.; Tanaka, T.; Ueki, K.; Maezawa, A.; Yano, S.; Naruse, T. *J. Leukoc. Biol.*, **1998**, *64*, 595.

Quantification of Intracellular SO_3^{2-} by MC-Gal

a



c



a: fluorescence calibration with MC-Gal

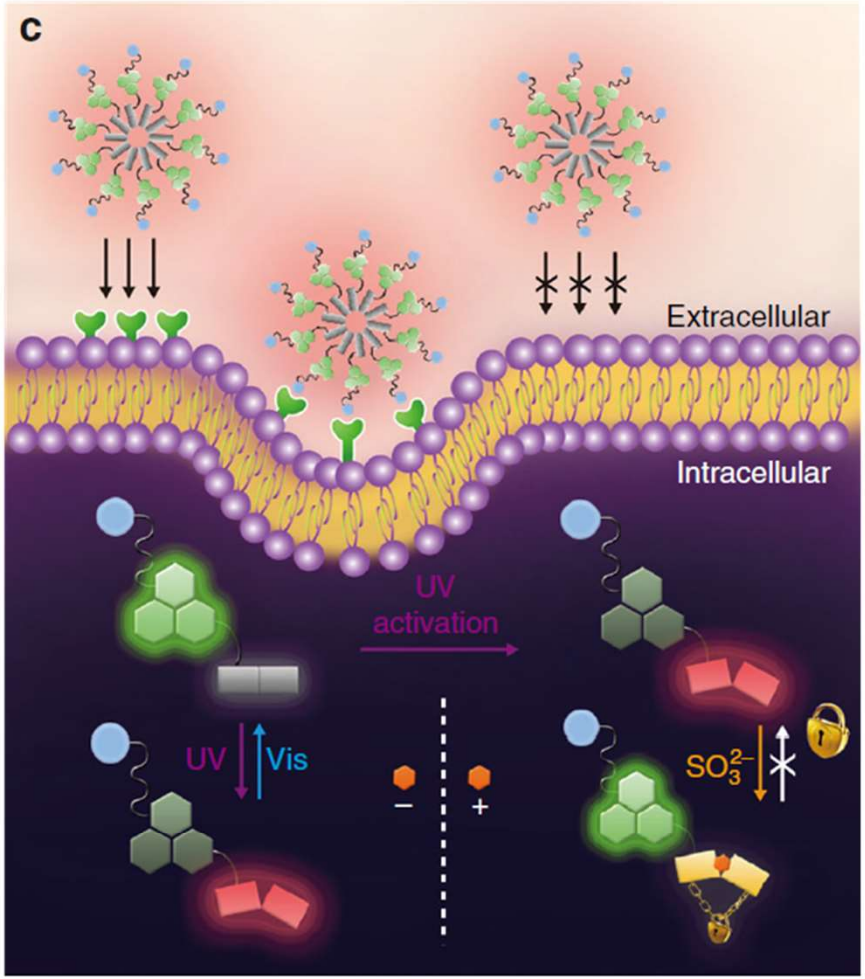
$[SO_3^{2-}]$ in cell lysate = 0.865 µM

c: ion chromatography (1:2 diluted)

$[SO_3^{2-}]$ in cell lysate = 0.70 µM

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Short Summary



The probe **actively localized** cells with a selective receptor via endocytosis.

ON/OFF of FRET was clearly controlled by photochromism *in vitro* and *in cell*.

-> Remaining possibility of duplexed switching (use of two color channels)

Activation of reactivity toward sulfite ion elicited under remote control.

-> Able to minimize interference of non-target molecules

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

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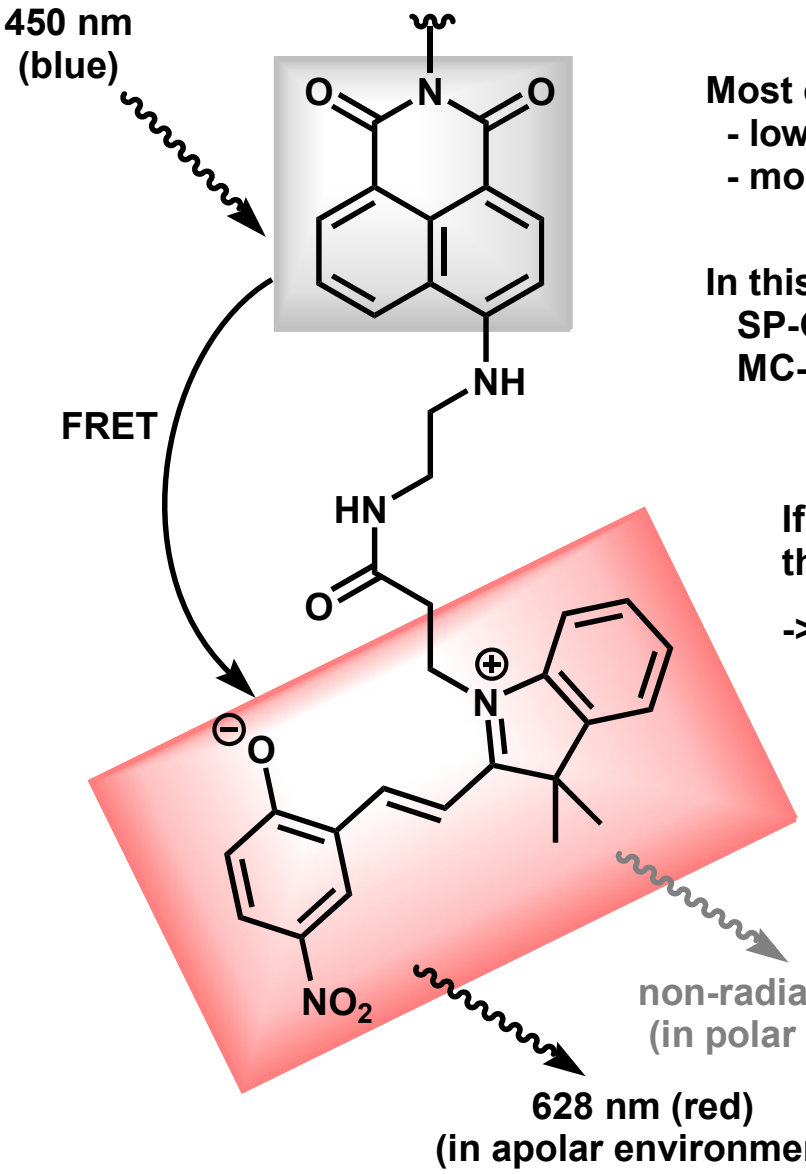
Photocontrolled Fluorescence “Double-Check” Bioimaging Enabled by a Glycoprobe−Protein Hybrid

Youxin Fu,[†,§] Hai-Hao Han,[†,§] Junji Zhang,[*,†] Xiao-Peng He,[*,†] Ben L. Feringa,[†,‡] and He Tian[*,†]

"Double-check" Bioimaging



Most of photochromophores are non-emissive because of ...
- **low fluorescence quantum yield**
- **molecular aggregation**

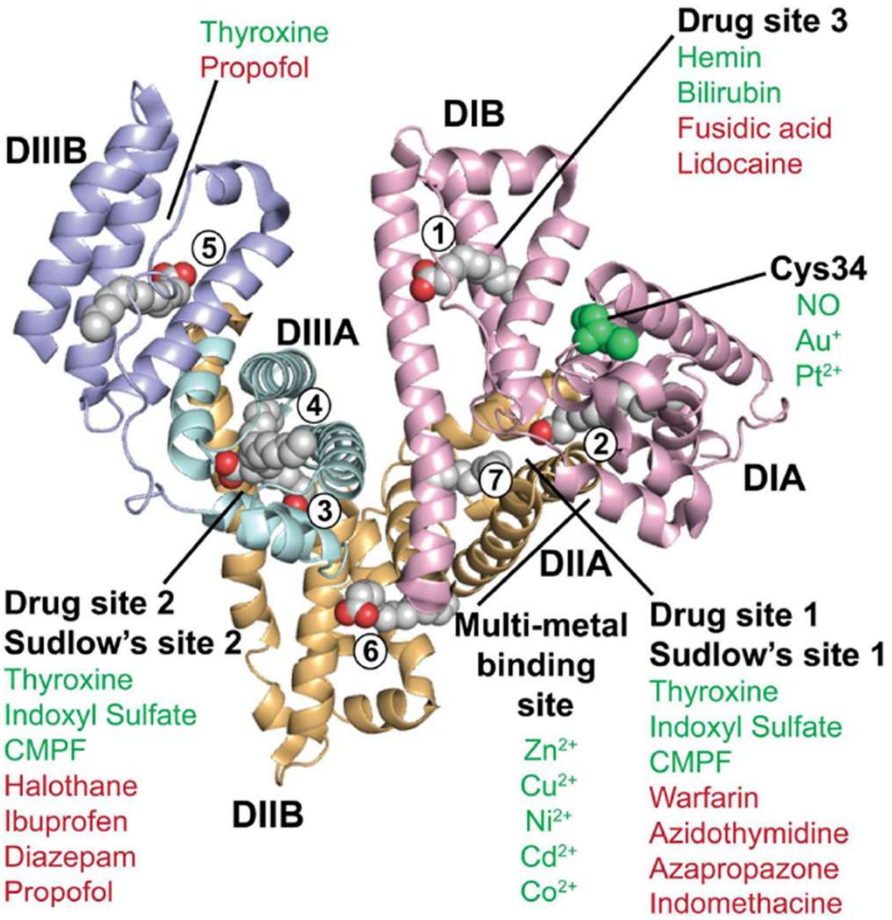
In this case...
SP-Gal: strong emission at 544 nm
MC-Gal: weak emission at 544 nm (non-FRET)
no emission at 628 nm (non-radiative relaxation)

If MC-Gal has strong emission at different wavelength to that of SP-Gal, "double-check" probing would be realized.

-> Host-guest strategy was employed to achieve apolar environment.

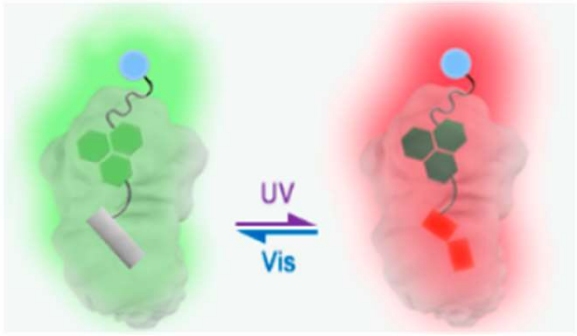
Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Human Serum Albumin (HSA)



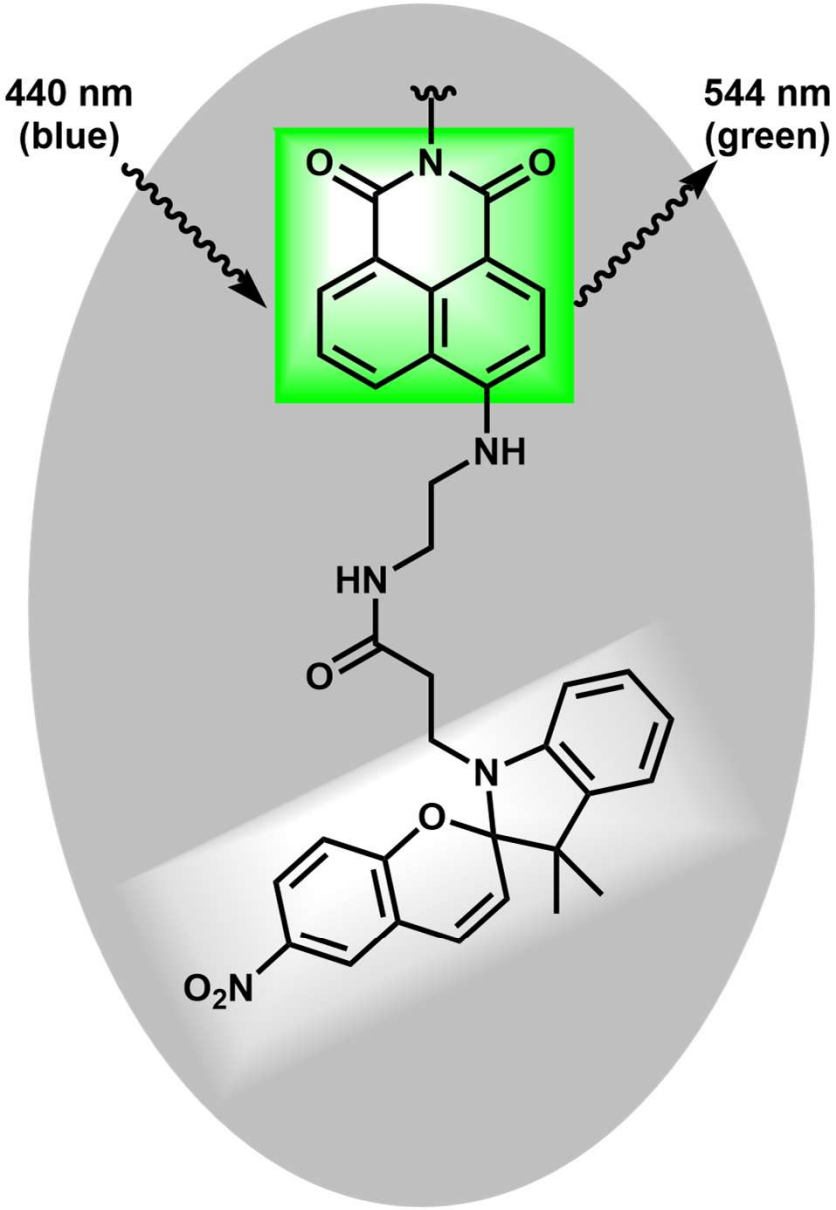
- produced by hepatocytes
- maintaining osmotic blood pressure
- antioxidant activity
- enzymatic property
- multi-carrier of insoluble/hydrophobic materials (endogenous/exogenous)

HSA is commonly used as biocompatible carrier for drug delivery.
-> Could it be applied to the strategy?

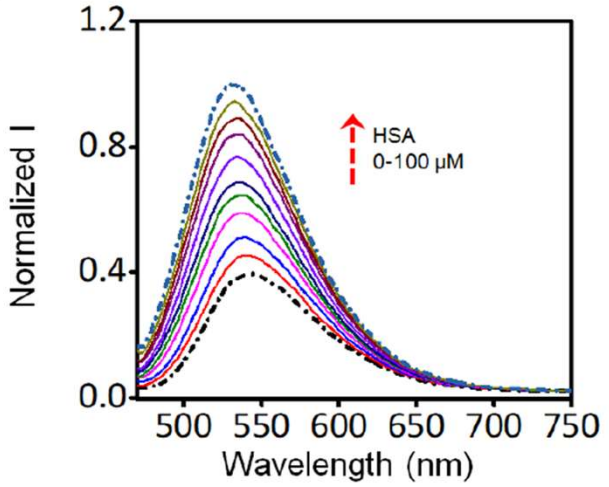


Sand, K. M. K.; Bern, M.; Nilsen, J.; Noordzij, H. T.; Sandlie, I.; Andersen, J. T. *Front. Immunol.*, **2015**, *5*, 682.

Binding of SP-Gal to HSA

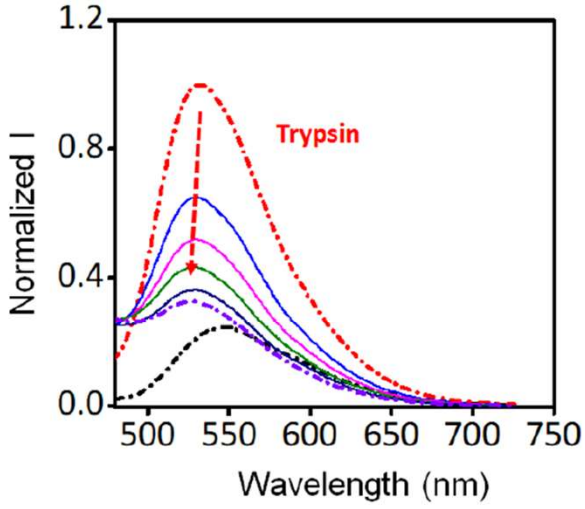


a



Fluorescence spectra of SP-Gal (5 μM)
with 0-100 μM HSA

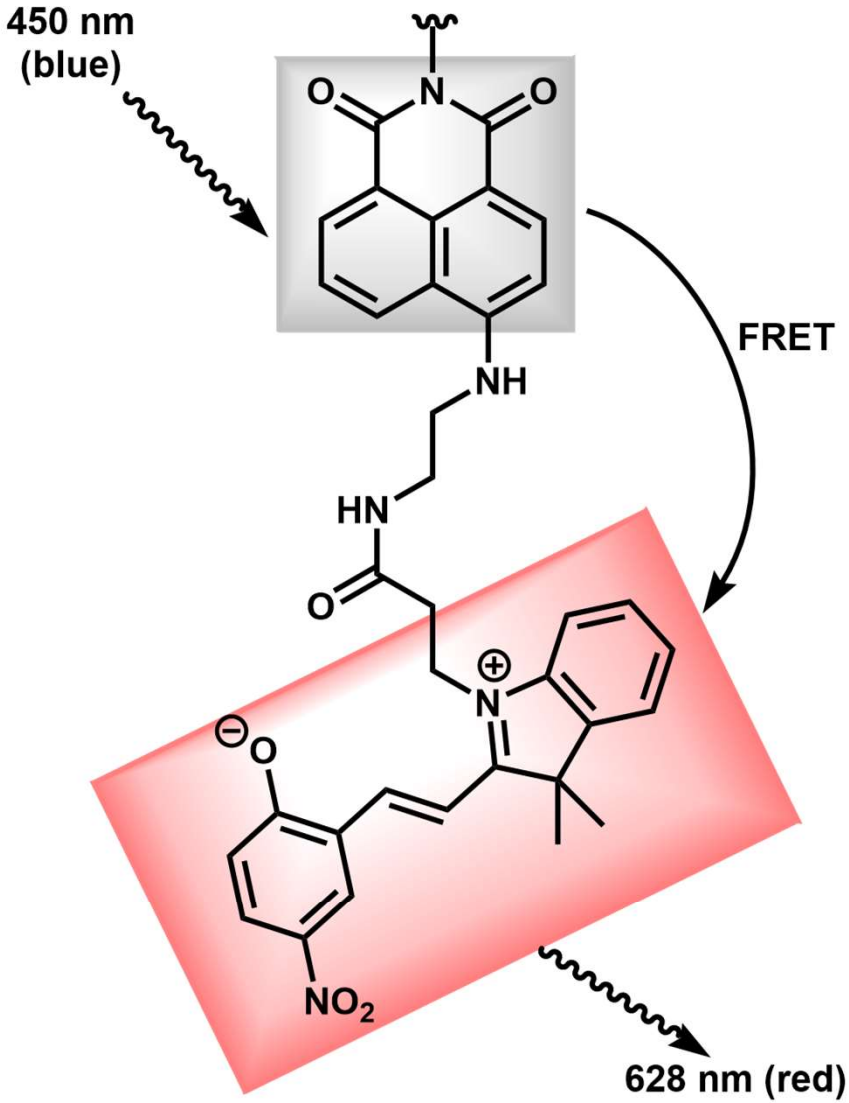
d

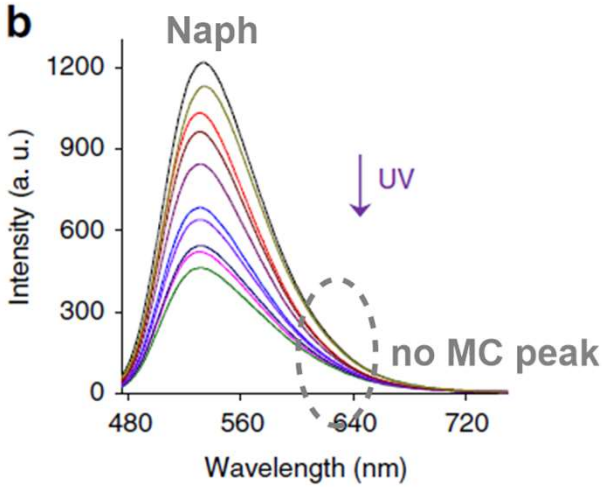


Fluorescence spectra of SP-Gal/HSA (5/100 μM)
with 0-600 μM Trypsin

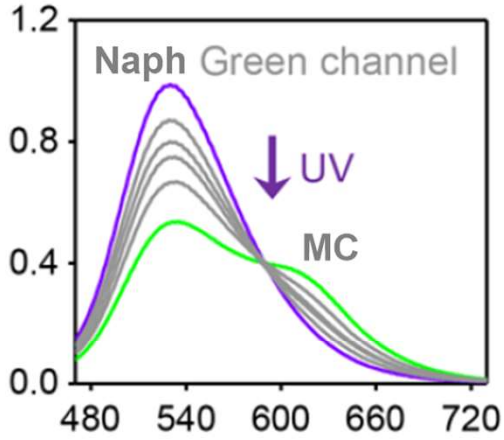
Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Photochromism of SP-Gal/HSA





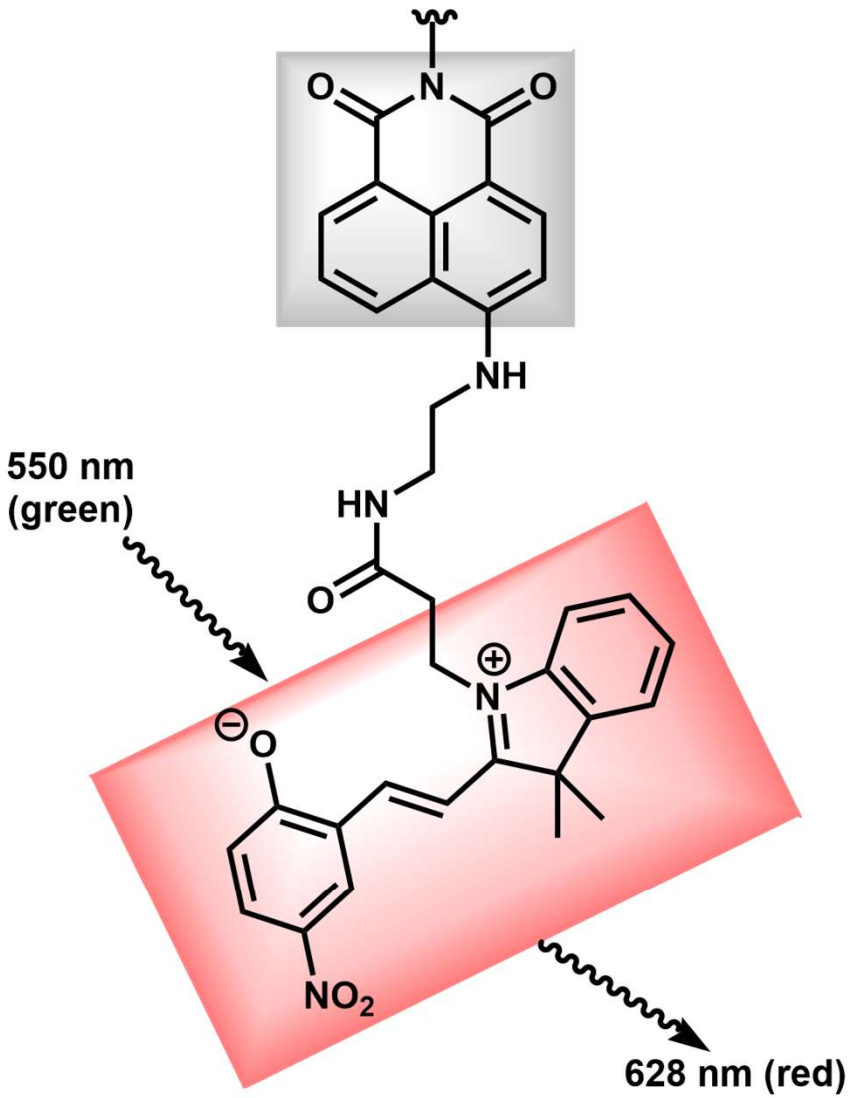
Fluorescence spectral change of **SP-Gal** (10 μM) upon UV (365 nm) irradiation excited by 440 nm[1)]

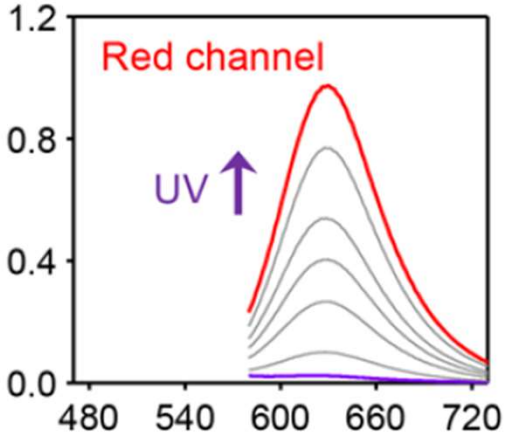


Fluorescence spectral change of **SP-Gal/HSA** (5/100 μM) upon UV (365 nm) irradiation excited by 450 nm[2)]

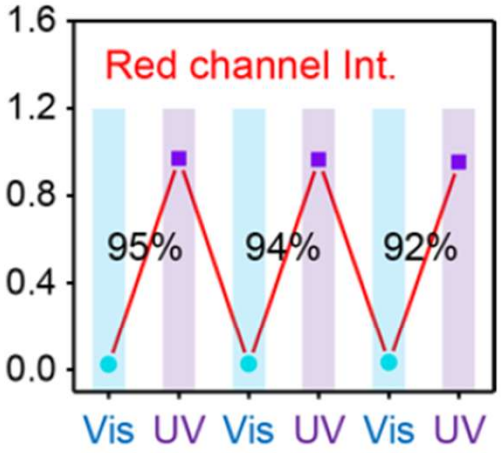
1) Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.
2) Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Photochromism of SP-Gal/HSA





Fluorescence spectral change of **SP-Gal/HSA** (5/100 μM) upon UV (365 nm) irradiation excited by 550 nm



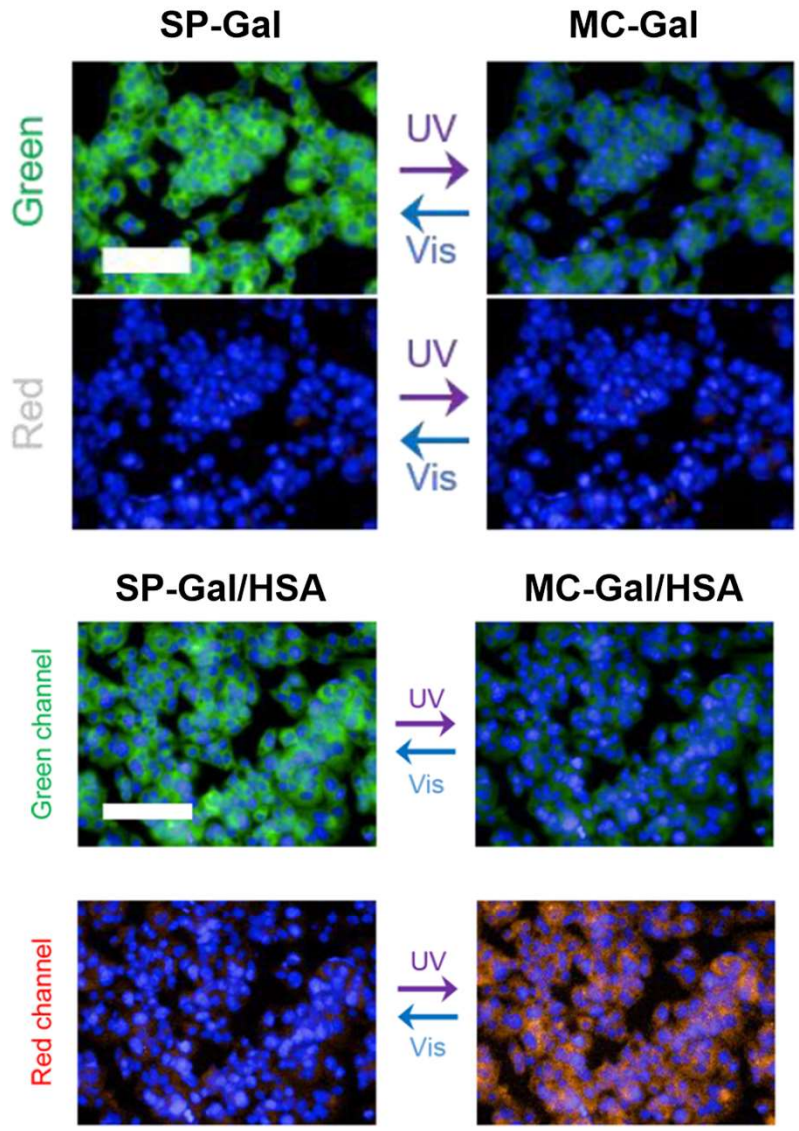
Fluorescence intensity cycling of **SP-Gal/HSA** (5/100 μM) at 628 nm excited by 550 nm

Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Intracellular Imaging by SP/MC-Gal/HSA

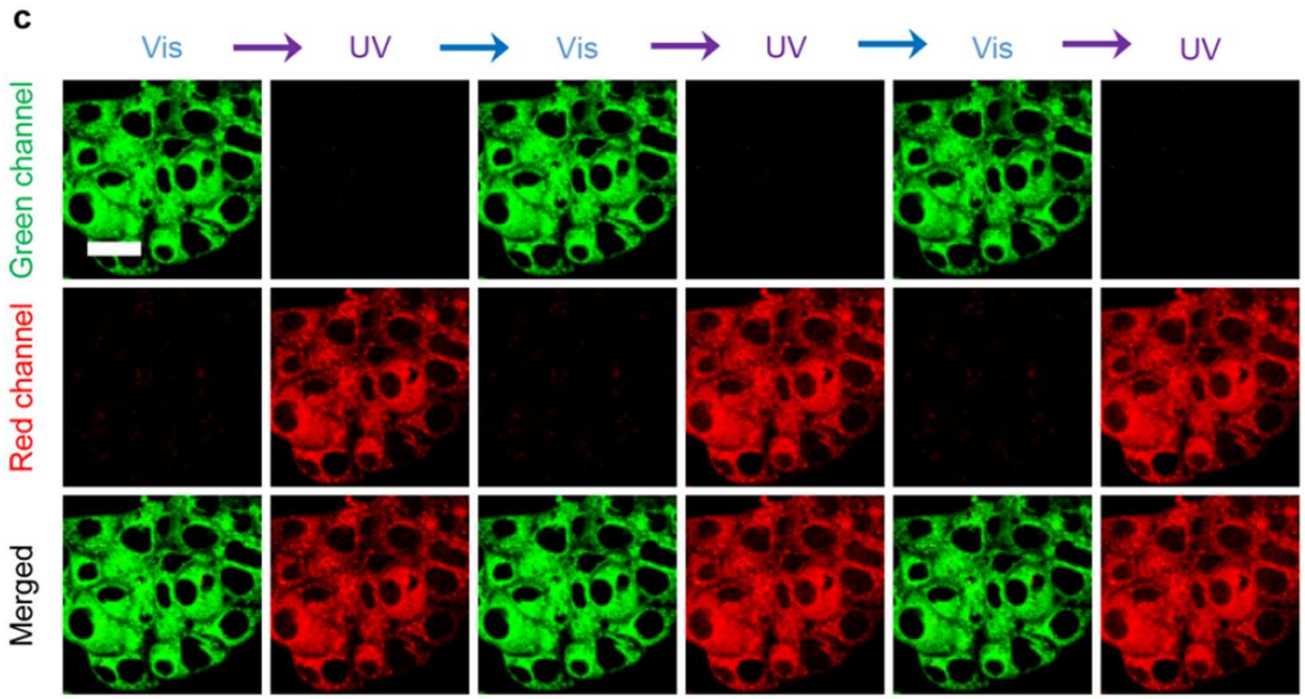
When cells were incubated solely with SP-Gal,
- **ON/OFF switch of green fluorescence (SP-Gal)**
- **no red fluorescence (MC-Gal)**

When cells were incubated with SP-Gal/HSA,
- **ON/OFF switch of green fluorescence (SP-Gal)**
- **OFF/ON switch of red fluorescence (MC-Gal)**



Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

UV/vis Cycling of Two Image Channels



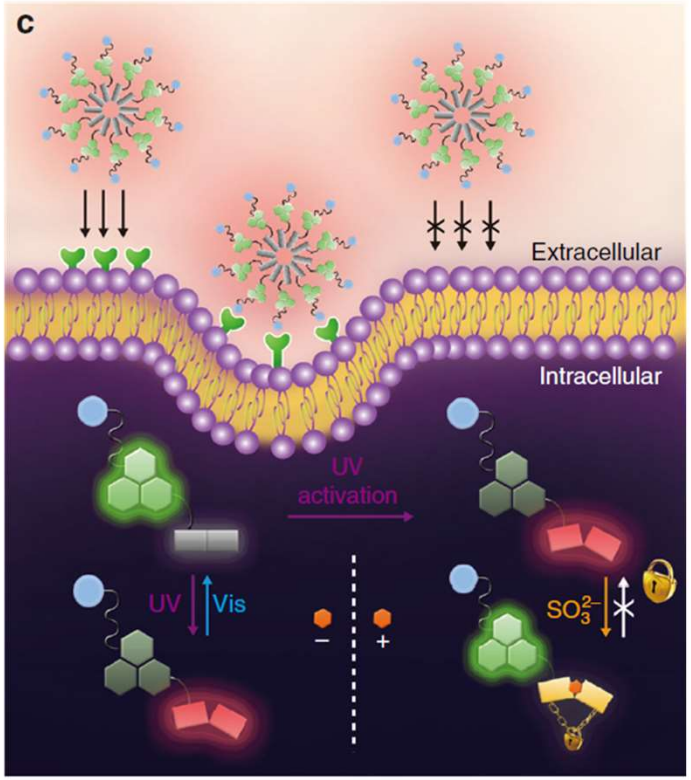
Hep-G2 cells with SP-Gal/HSA upon UV/vis irradiation
Green: Ex. 440 nm, Em. 535 nm; Red: Ex. 579 nm, Em. 603 nm

No yellow region on merged image was observed.
-> Reversible "blinkings" in two fluorescent channels were precisely controlled in target cells.

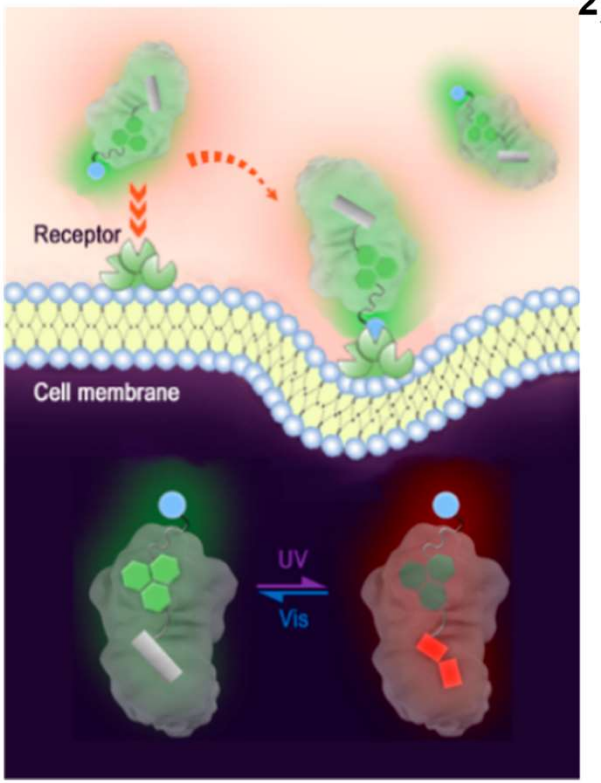
Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Summary

1)



2)



Achievements:

proof-of-concept of fluorophore-photochromophore conjugate for FRET switching
recovery of fluorescence by target capture
enhancement of quantum yields using HSA

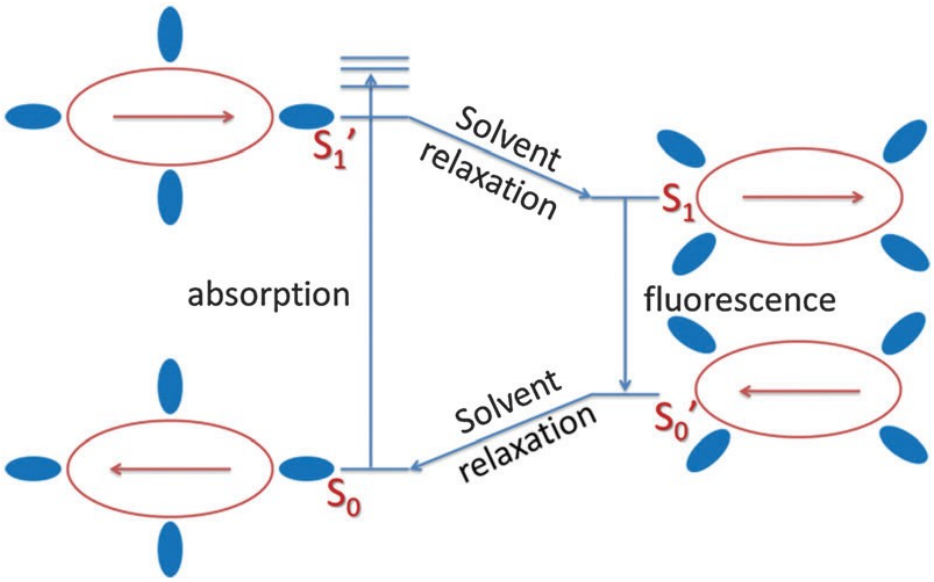
Future perspectives:

conjugation with other ligands - localization to cells with other receptors
third fluorescence after capture of target

1) Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.
2) Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Appendix

Solvent Effect on Fluorescence



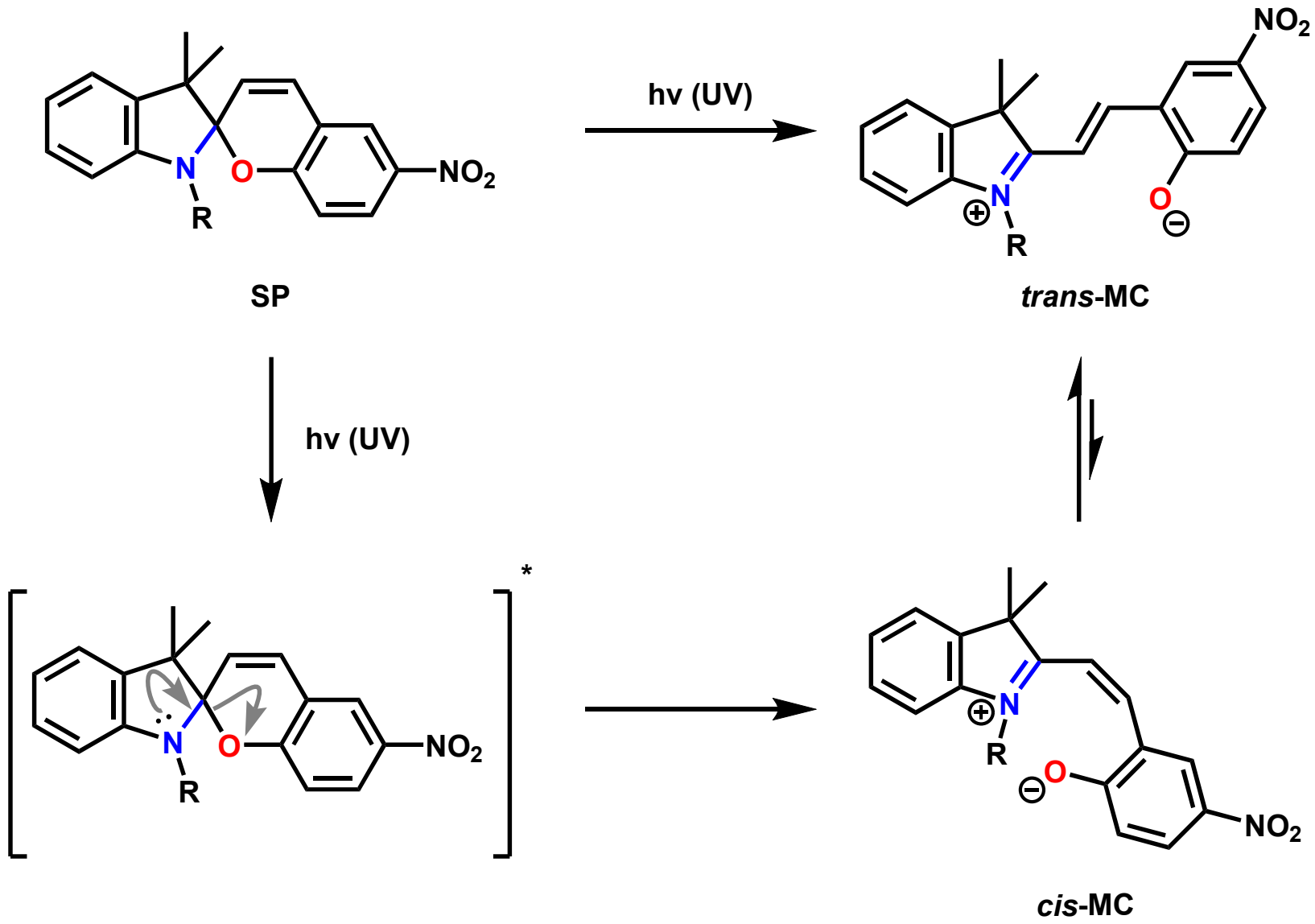
Shift of fluorescence spectrum

increase of non-radiative transition

(aggregation of dye molecules)

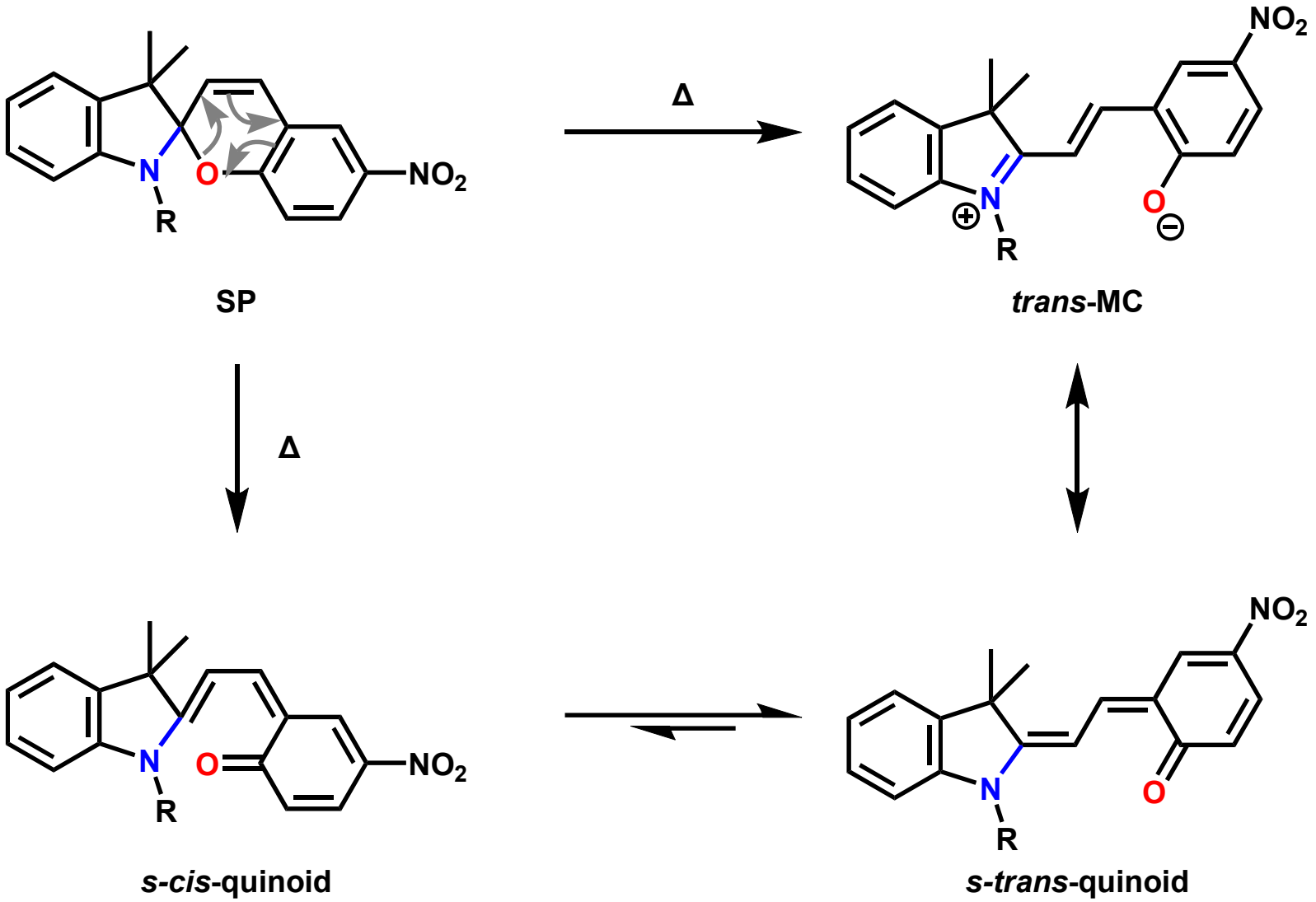
Yang, Z.; Cao, J.; He, Y.; Yang, J. H.; Kim, T.; Peng, X.; Kim, J. S. *Chem. Soc. Rev.*, **2014**, *43*, 4563.

Mechanism of Isomerization by Light



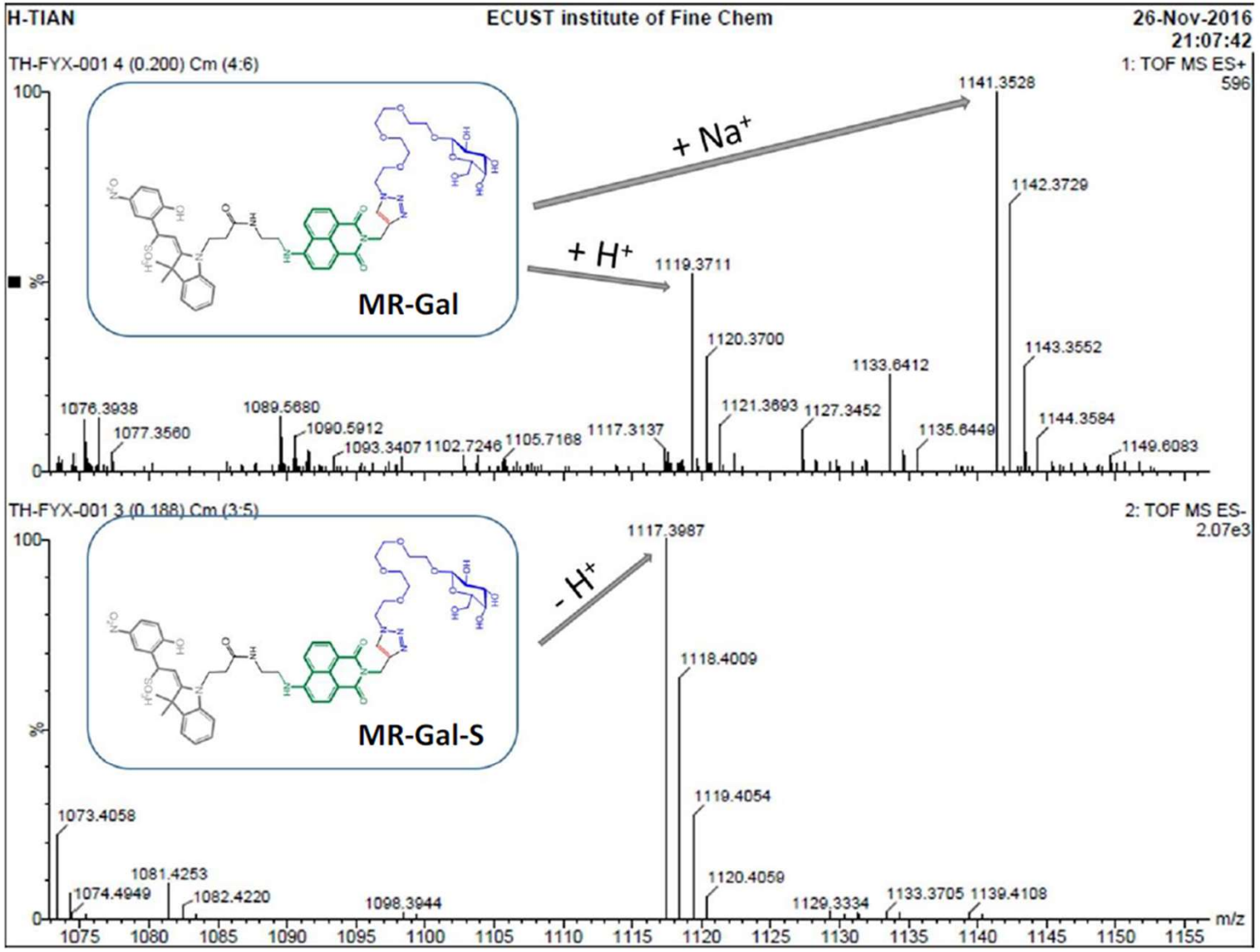
Klajn, R. *Chem. Soc. Rev.*, **2014**, *43*, 148.

Mechanism of Isomerization by Heat



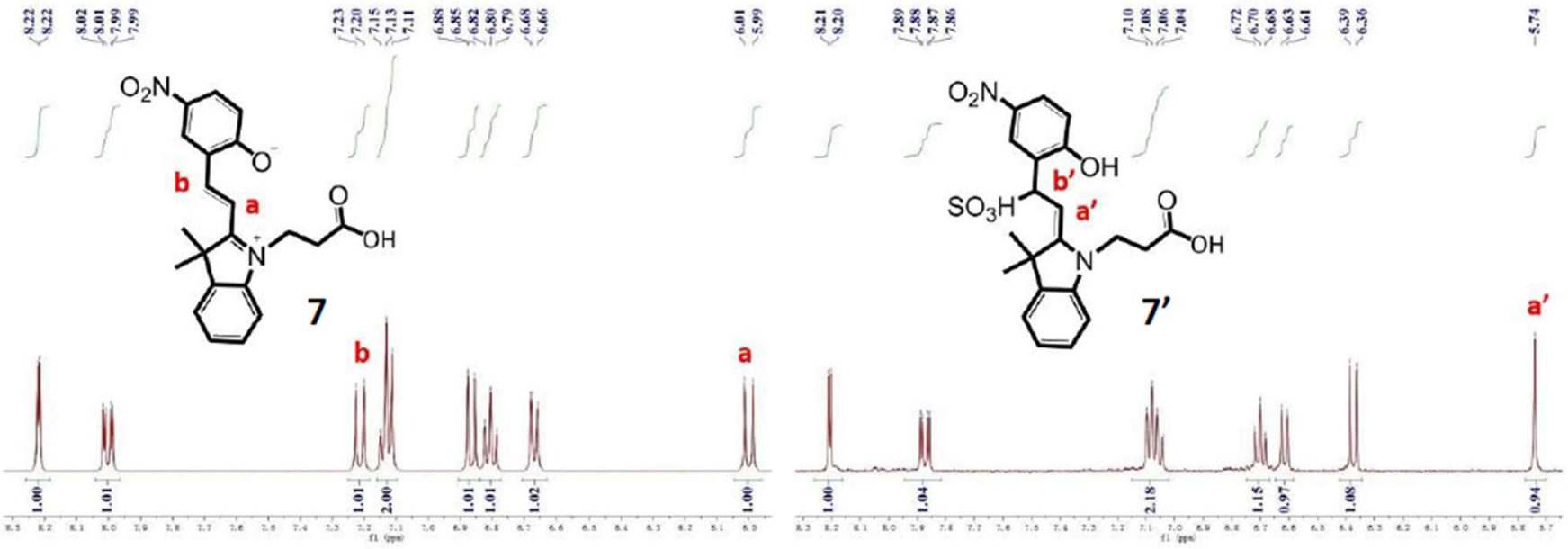
Klajn, R. *Chem. Soc. Rev.*, **2014**, *43*, 148.

Structure Determination of MC-Gal-SO_3^{2-}



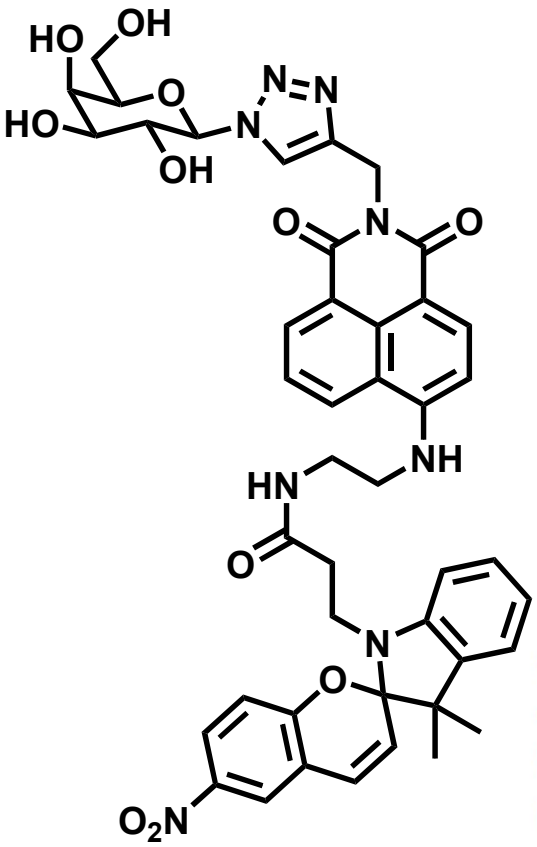
Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Structure Determination of MC-Gal-SO_3^{2-}

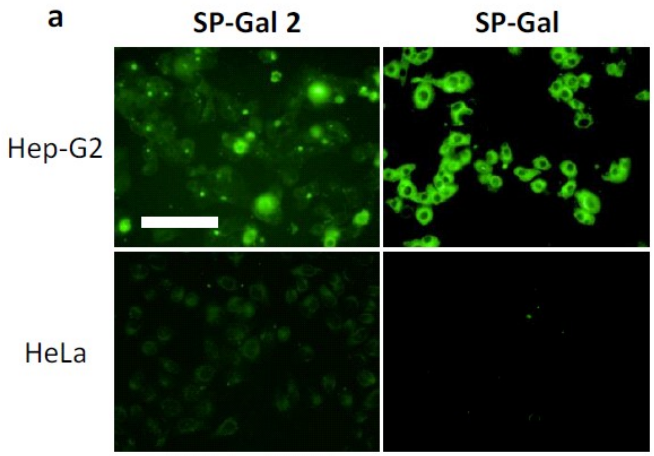


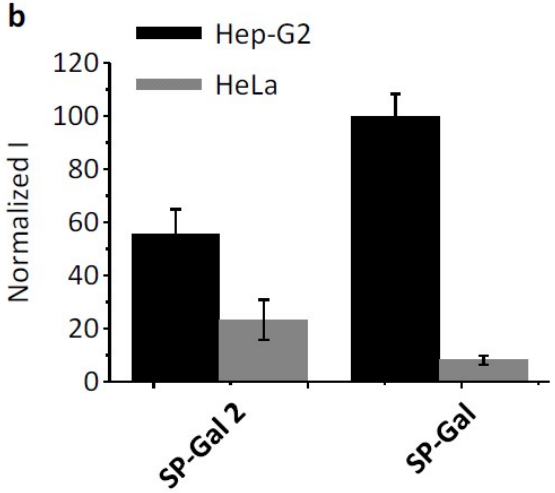
Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

PEG Linker



SP-Gal 2
(no PEG linker)





Supplementary Figure 8. (a) Fluorescence imaging of **SP-Gal 2** (20 μM) *vs.* **SP-Gal** (20 μM) for two different human cancer cell lines (Hep-G2 = human liver cancer; HeLa = human cervical cancer). (b) Fluorescence quantification of **SP-Gal 2** (20 μM) *vs.* **SP-Gal** (20 μM) for different cells. For all fluorescence images, the excitation wavelength was 440 nm and emission channel 450-550 nm (scale bar: 100 μm, which is applicable to all images; the error bar represents s.d. (n = 3)).

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Biospecificity of MC-Gal

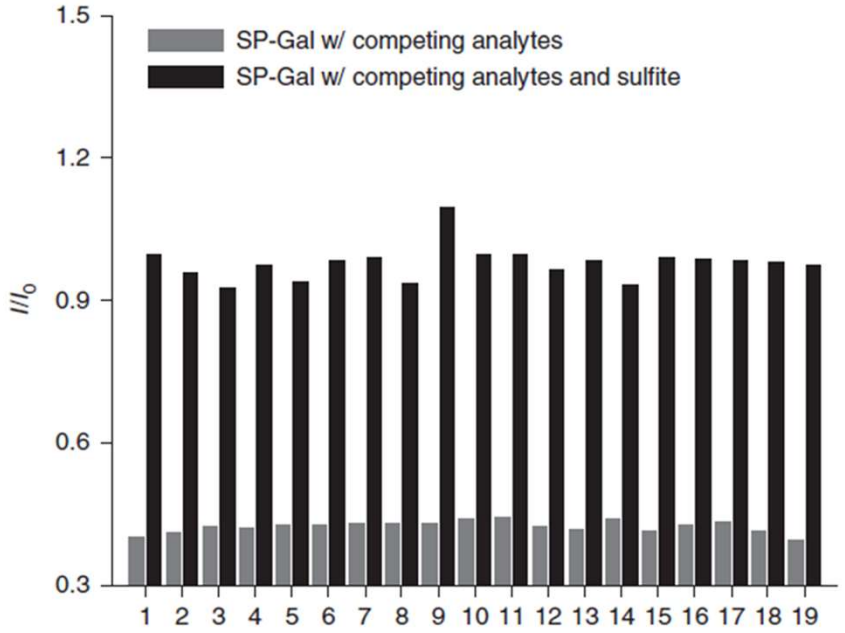
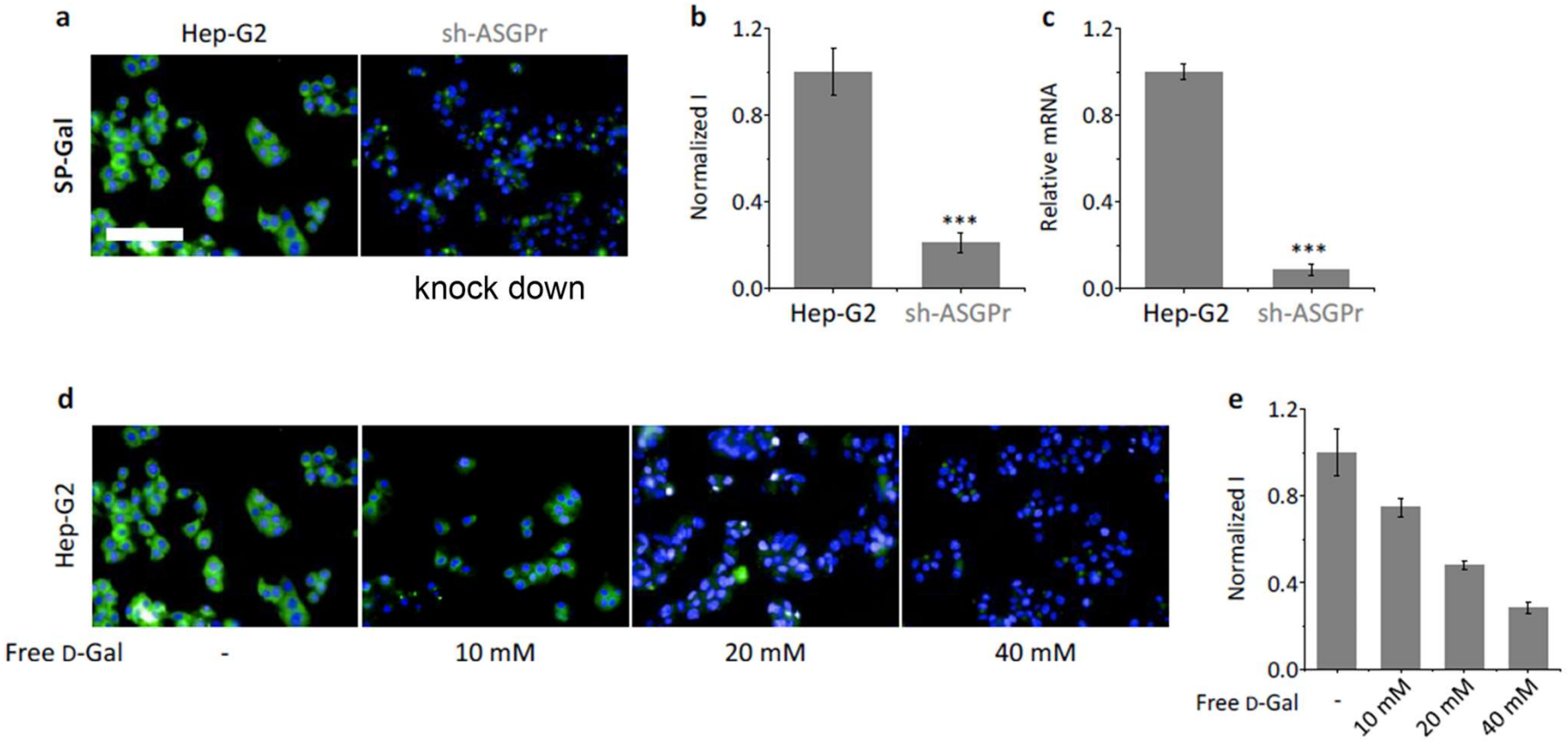


Fig. 5 Biospecificity of photochromic fluorescent glycoprobe. The fluorescence change of MR-Gal (10^{-5} M) with a range of competing analytes (10^{-3} M) and the mixture of the analytes (10^{-3} M) with sulfite (5×10^{-5} M) in phosphate buffered saline (PBS, 0.01 M, 1% DMSO, pH 7.4) at 298 K (excitation wavelength: 450 nm), where I_0 and I are the fluorescence of the initial spiropyran state and that of the UV-activated merocyanine state, respectively. Competing analytes 1–19: 1: Blank (probe alone), 2: F^-; 3: Cl^-; 4: Br^-; 5: I^-; 6: NO_3^-; 7: NO_2^-; 8: CH_3COO^-; 9: HCO_3^-; 10: SO_4^{2-}; 11: $S_2O_3^{2-}$; 12: PO_4^{3-}; 13: CO_3^{2-}; 14: Cys; 15: Hcy; 16: GSH; 17: HPO_4^{2-}; 18: $H_2PO_4^-$; 19: N_3. We note that the physiologic concentration of common thiols and nucleophiles such as Hcy, Cys is in the range of 10–200 μM[49–51], which is well-below the concentration used in this experiment

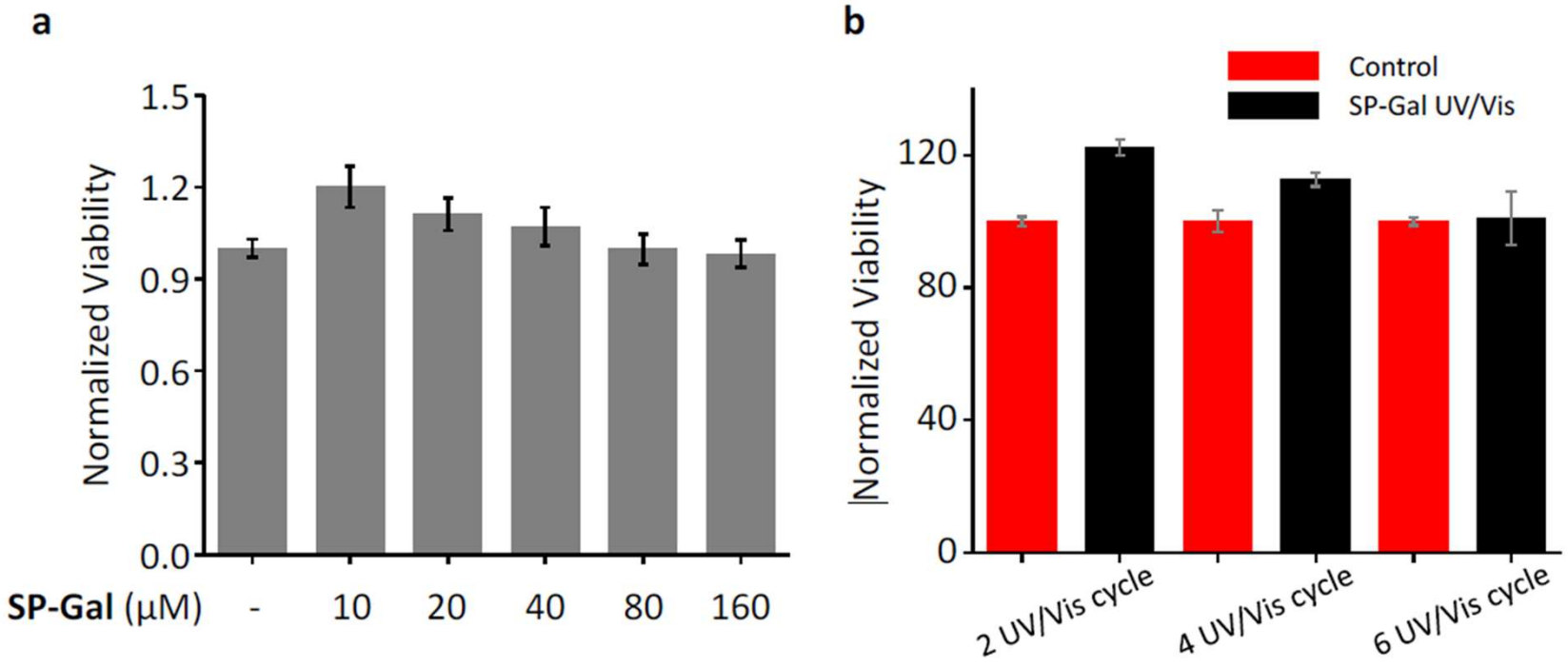
Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Glycoprotein Receptor



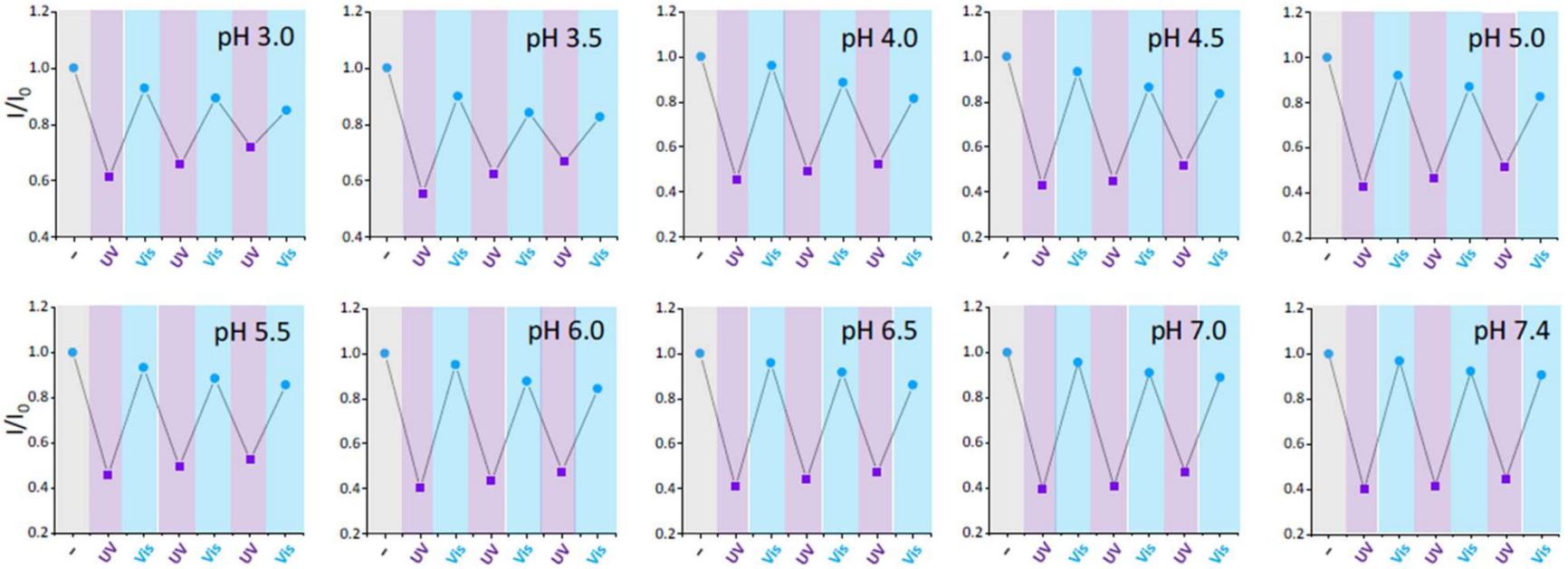
Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Cell Viability



Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

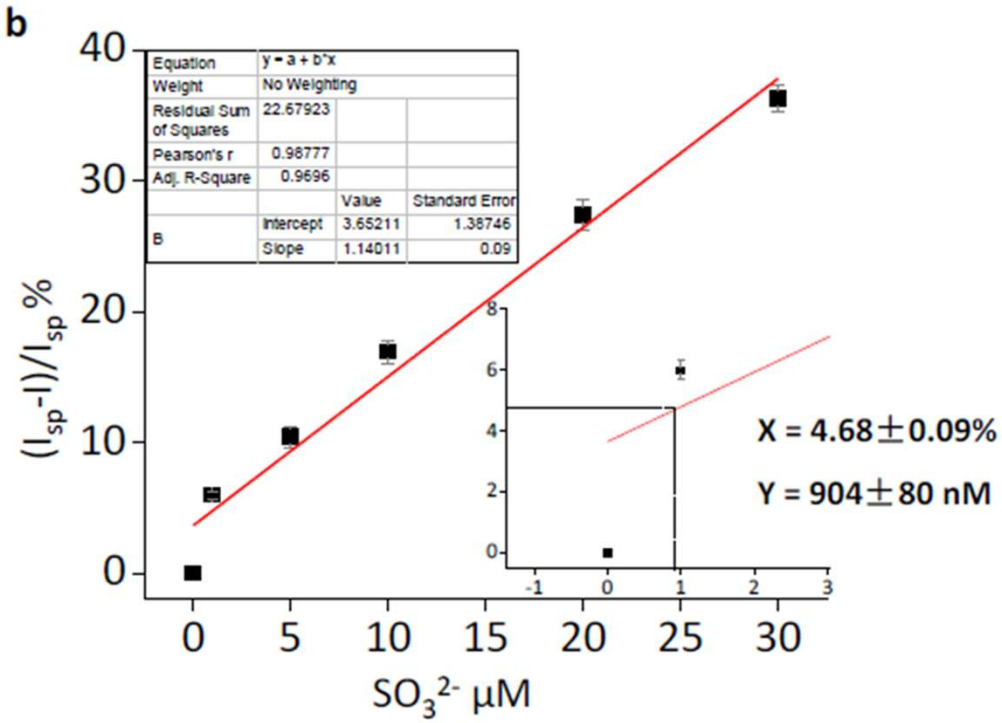
Photoswitch with Different pH



Supplementary Figure 11. Photoswitching of SP-Gal with different pH. Stock solution of **SP-Gal** (1 mM) was prepared in DMSO. Test solutions of **SP-Gal** (10 μM) were prepared in PBS (0.01 M, 1% DMSO) with different pH (3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0 and 7.4). I_0 and I are the initial fluorescence intensity of **SP-Gal** and that of the corresponding **SP-Gal/MR-Gal** upon alternate UV-Vis irradiation, respectively.

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Quantification of Endogenous SO_3^{2-} by MC-Gal



Quantification of lipopolysaccharide (LPS) induced endogenous sulfite in live Hep-G2 cells by fluorescence calibration, where I_{sp} and I are the initial fluorescence intensity of SP-Gal and that of MR-Gal (converted by UV) after reaction with various concentrations of sulfite, respectively.

Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.

Quantum Yields

Supplementary Table 1. Fluorescence quantum yield (Φ_F), photochromic quantum yield (Φ_P) and fluorescence lifetime (τ) of SP-Gal and MR-Gal. [1)]

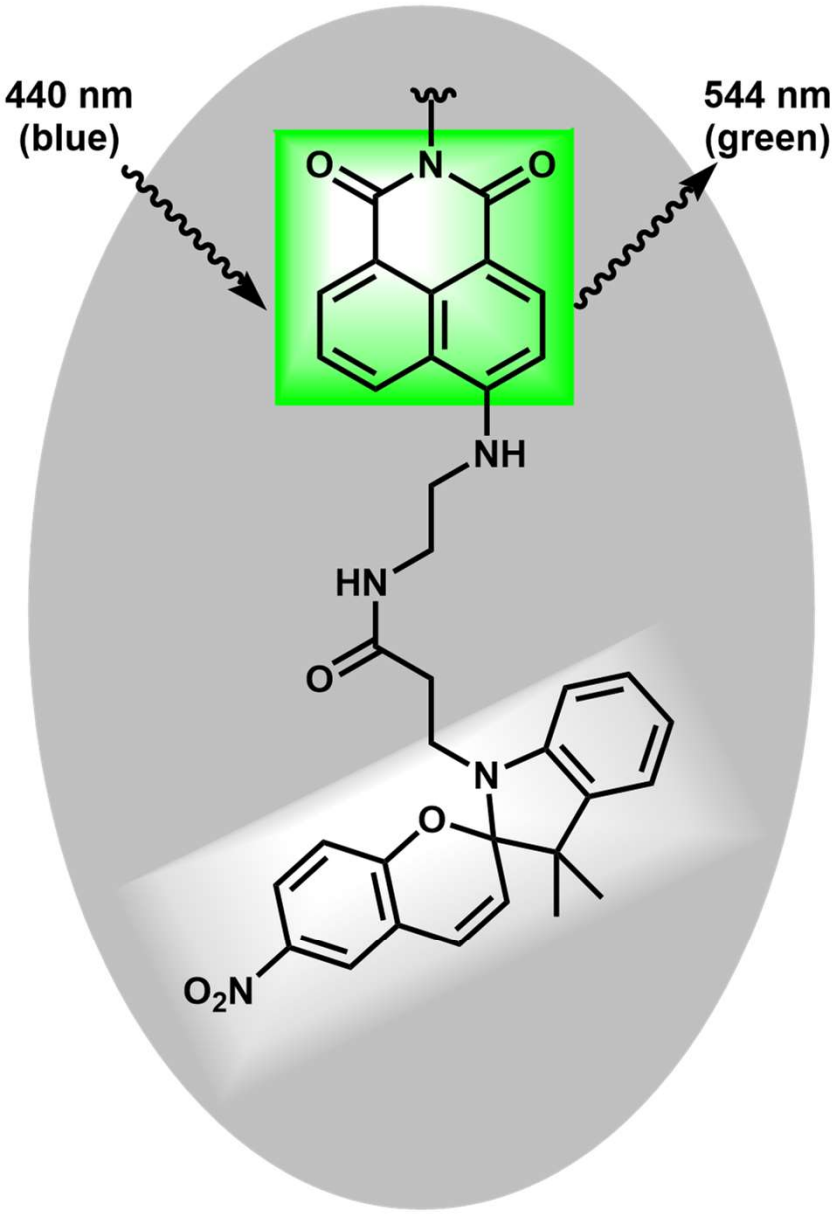
	SP-Gal	MR-Gal
Φ_F (%)	32.10	7.81
Φ_P (%)	5.78	14.41
τ (ns)	4.14	1.41

Table S2. Photochromic quantum yields (Φ_P) of **Gal-NSp/HSA** and **Gal-NMr/HSA** in phosphate buffered saline (containing 0.5% DMSO (v/v), pH 7.4). [2)]

	SP-Gal	MR-Gal
Φ_P (%)	7.25	17.21

1) Zhang, J.; Fu, Y.; Han, H.-H.; Zang, Y.; Li, J.; He, X.-P.; Feringa, B. L.; Tian, H. *Nat. Commun.* **2017**, *8*, 987.
2) Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.

Binding Site of SP-Gal



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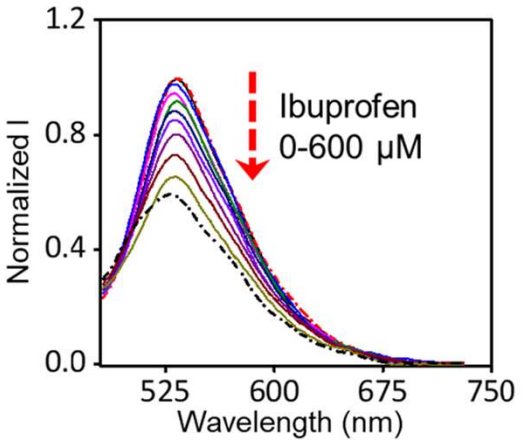
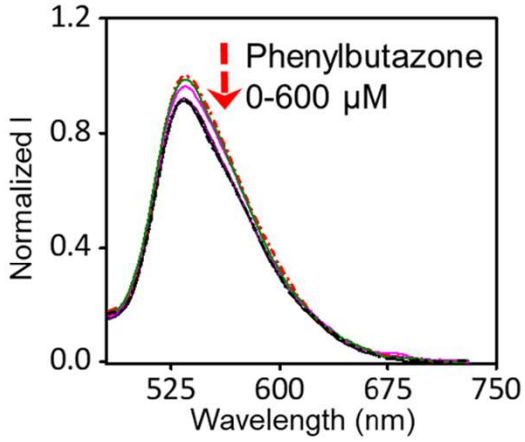


Fluorescence spectra of SP-Gal/HSA (5/100 μM)
with 0-600 μM Ibuprofen (known to bind IIIA domain)

b



Fluorescence spectra of SP-Gal/HSA (5/100 μM)
with 0-600 μM Phenylbutazone (known to bind IIA domain)

Fu, Y.; Han, H.-H.; Zhang, J.; He, X.-P.; Feringa, B. L.; Tian, H. *J. Am. Chem. Soc.*, **2018**, *140*, 8671.