Fluorescent Formaldehyde Probe

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20240201

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  - Formimine-based
  - Hydrazine-based
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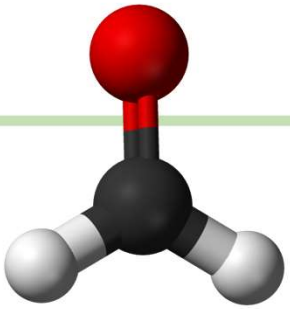
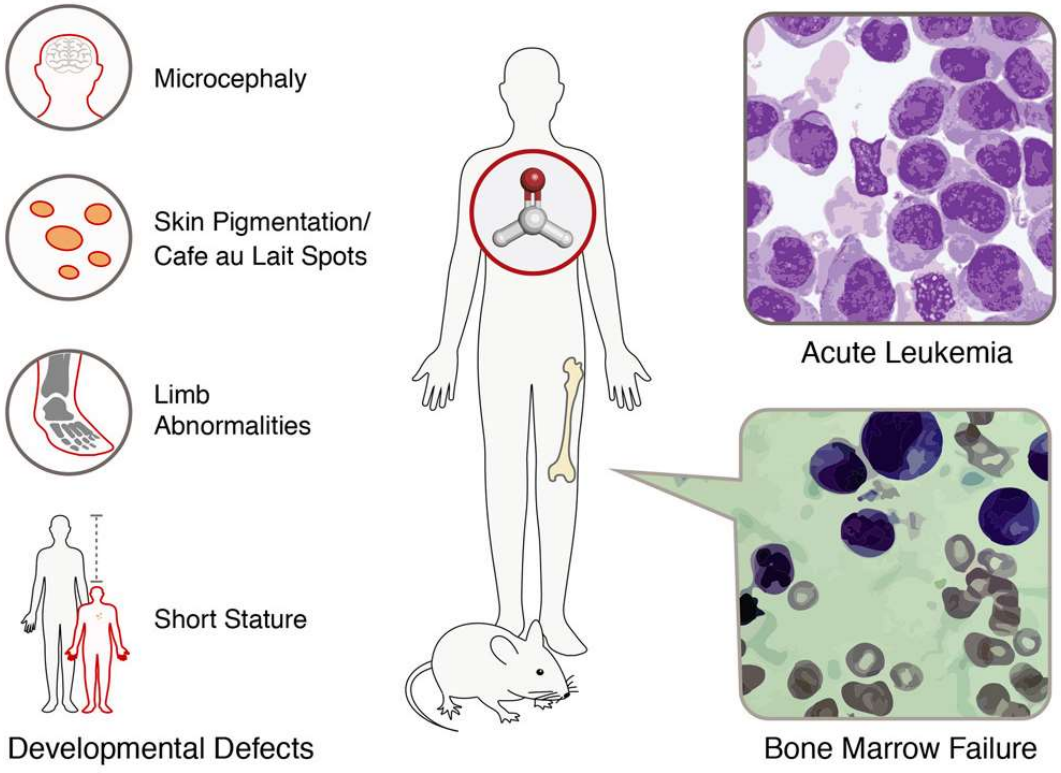
- Summary

Formaldehyde(FA)

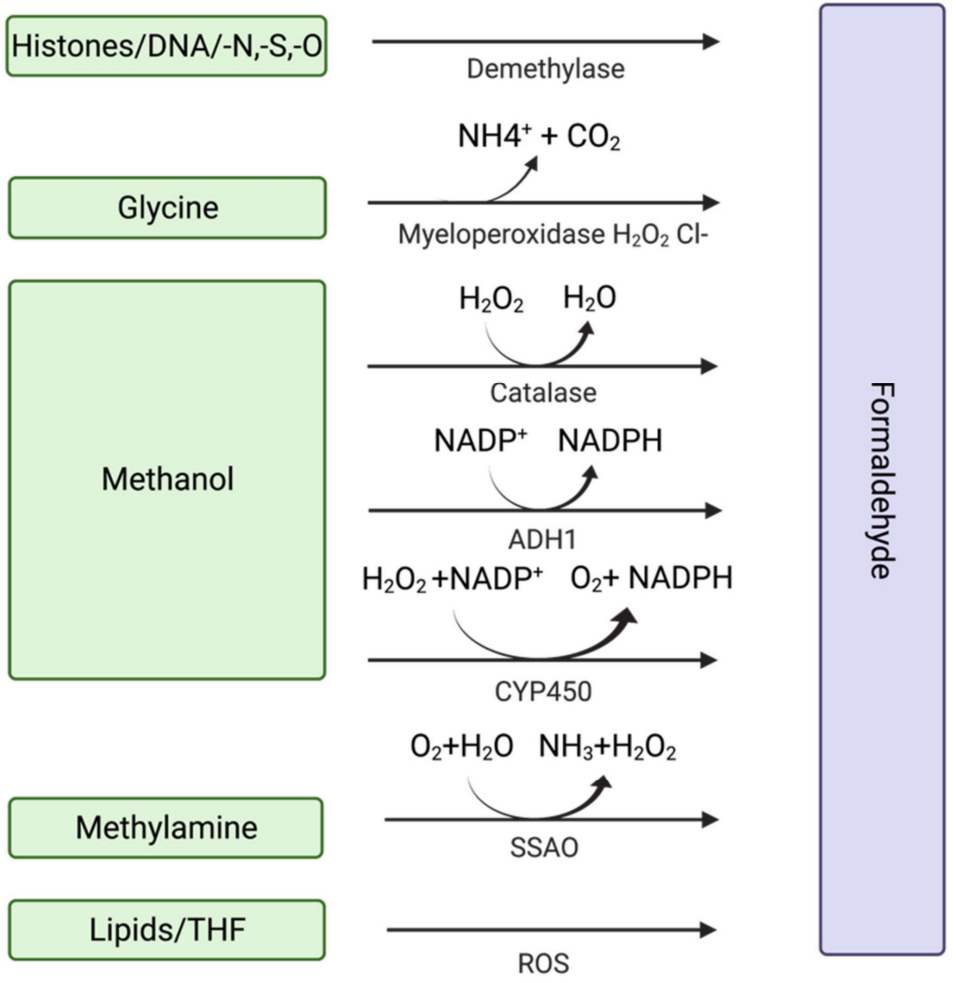
- Strong Electrophile
- Reactive Carbonyl Species



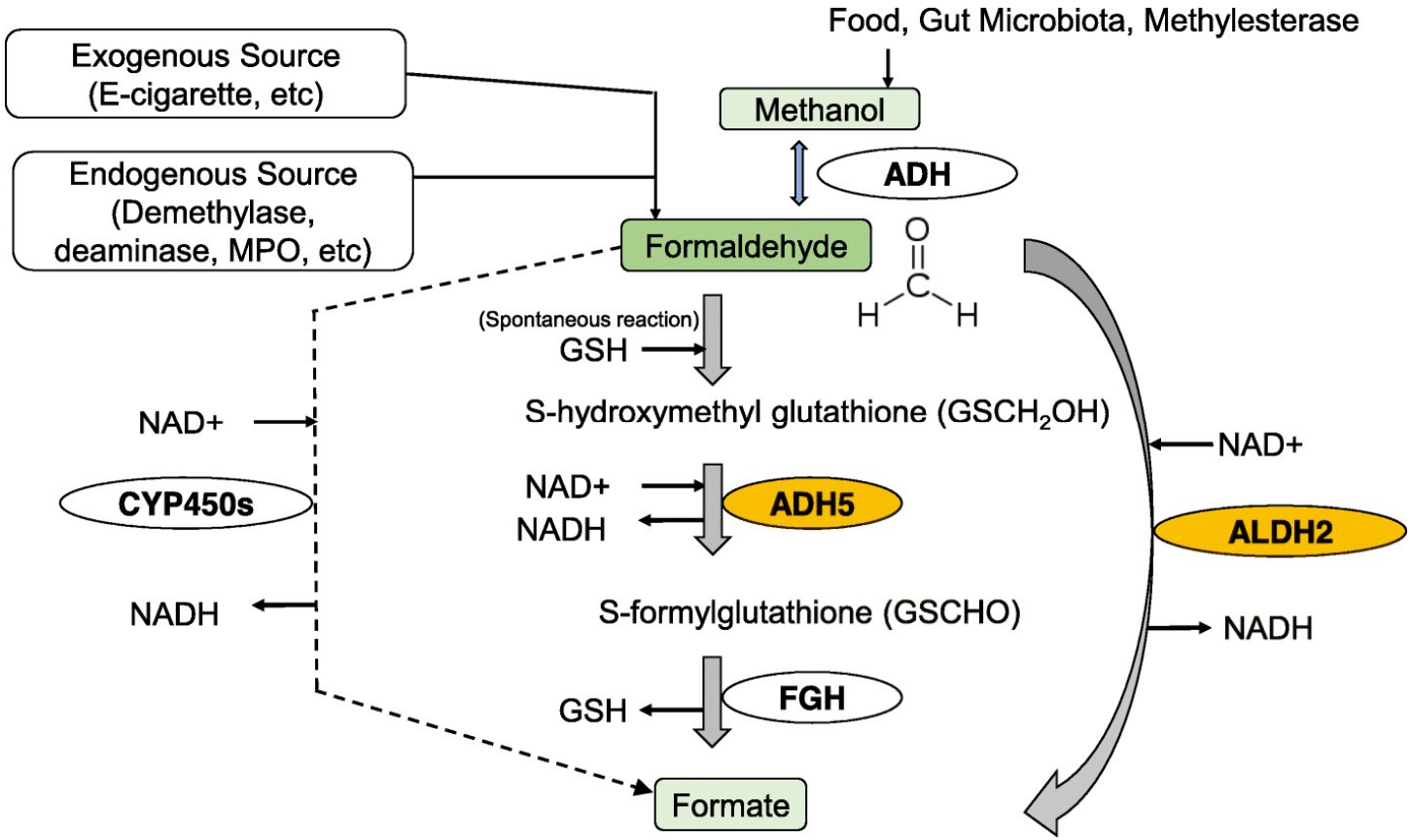


Endogenous Formaldehyde producing

- N-methyl group demethylase
- Serine hydroxymethyltransferase (SHMT)
- Dimethylglycine dehydrogenase
- Oxidative demethylation enzymes
- P450 oxidase
- Semicarbazide-sensitive amine oxidases (SSAOs)
- Lipid oxidation enzymes



Decomposing

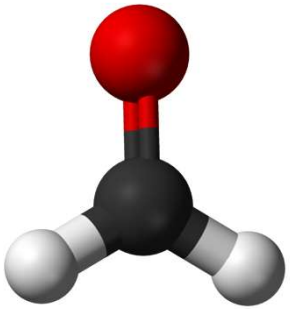


FA is mainly decomposed by ADH5 & ALDH2.

Short Summary

Normal

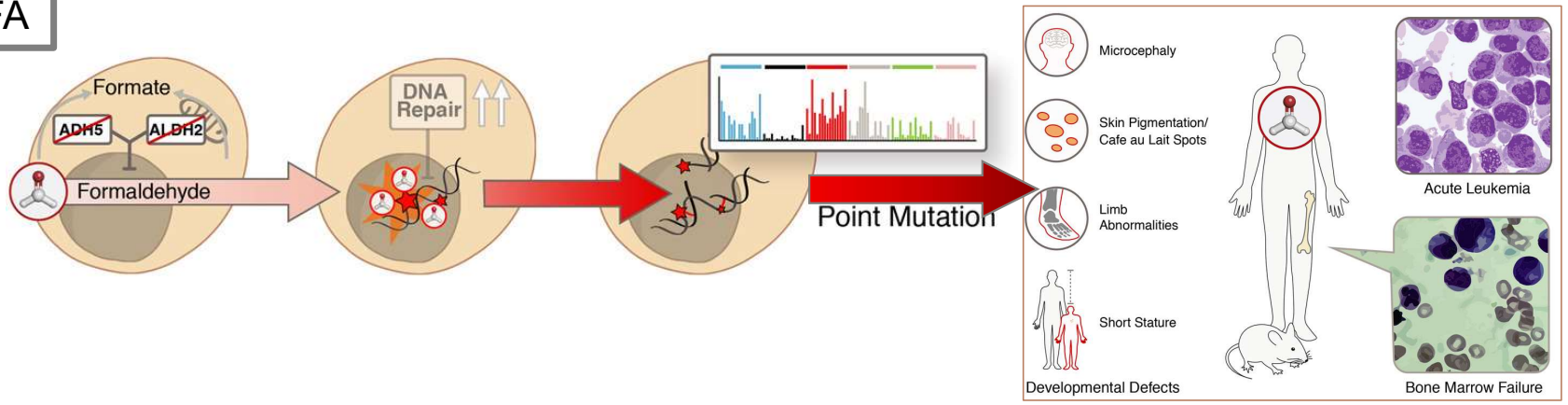
Demethylase, Catalase, ADH1, CYP450, SSAO, ROS, SHMT, Dehydrogenase



ADH5 **ALDH2**

Formate

Elevated FA



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  - Hydrazine-based
    - Na-FA
  - 2-aza-Cope Rearrangement -based
    - FAP-1
    - FP1
    - FA Trigger
    - RFAP
      - Formaldehyde regulates S-adenosylmethionine biosynthesis
- Summary

Detection of FA

- Colormetric assays
- HPLC
- GC
- Radiometric assays
- Mass spectrometry

× Lack sensitivity
× Invasive destruction
→ loss of spatiotemporal information

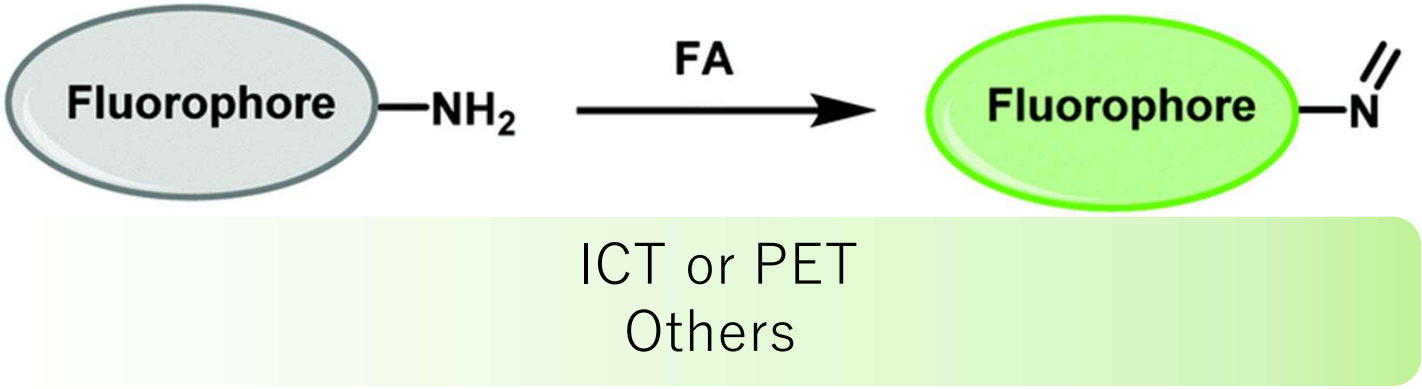
- Noninvasive
- High sensitivity

Fluorescent Formaldehyde-Probe

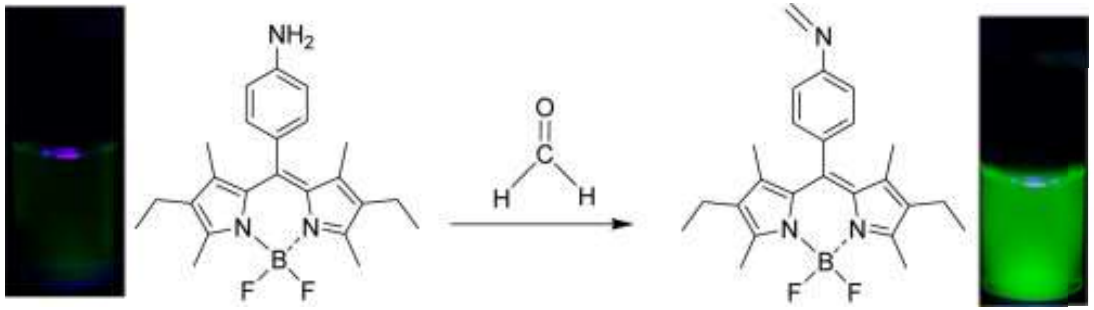
Design of Formaldehyde Probe

- Formimine-based
- Hydrazine-based
- 2-Aza-Cope rearrangement

Formimine-based Probe

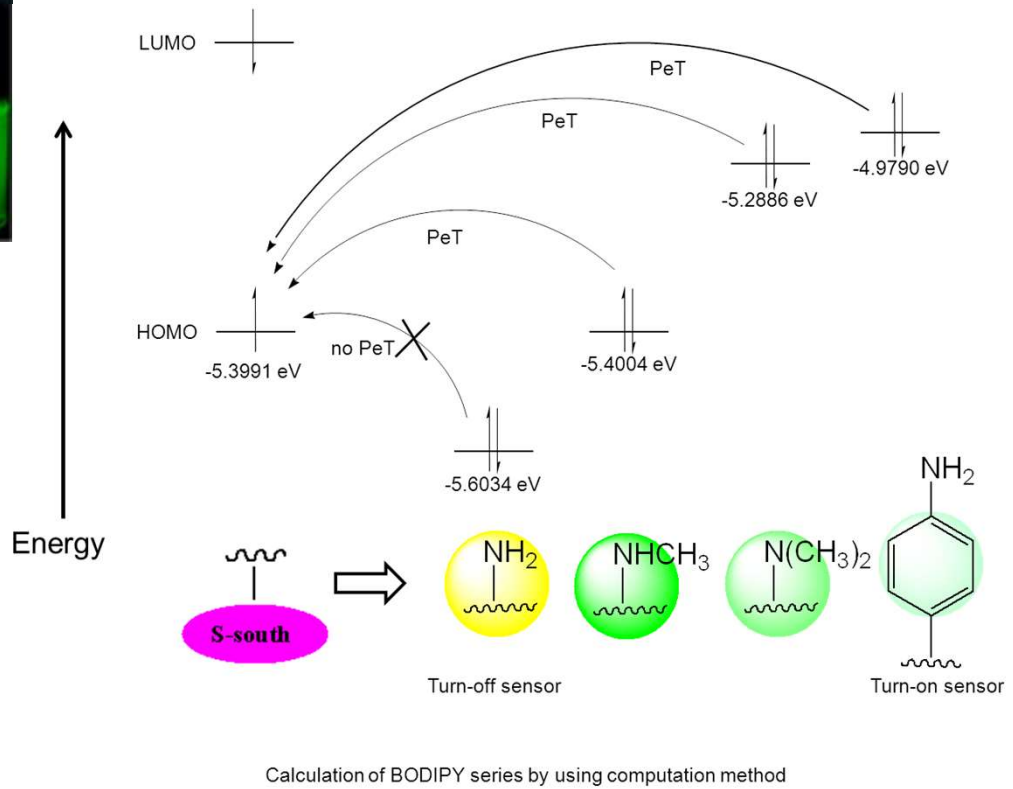


AnB



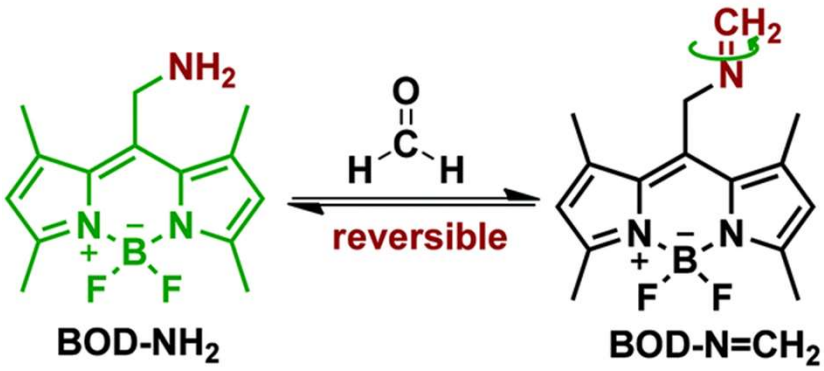
- First Formaldehyde Probe
- Based on theoretical calculations

✓ FA(37%) methanol at pH8. (5μM)



Calculation of BODIPY series by using computation method

BOD-NH $_2$



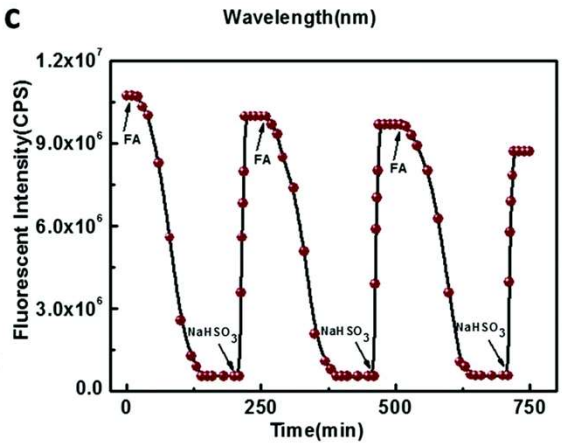
- C＝N isomerization

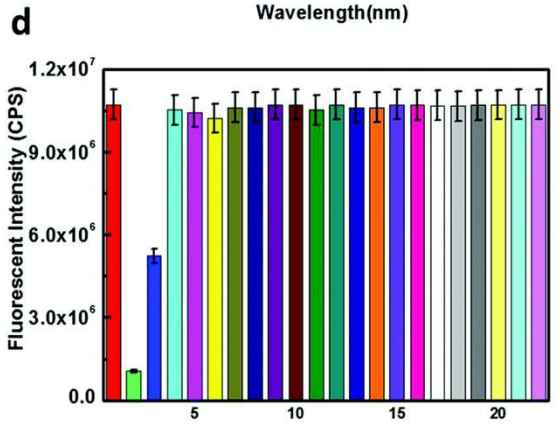
✓Good selectivity

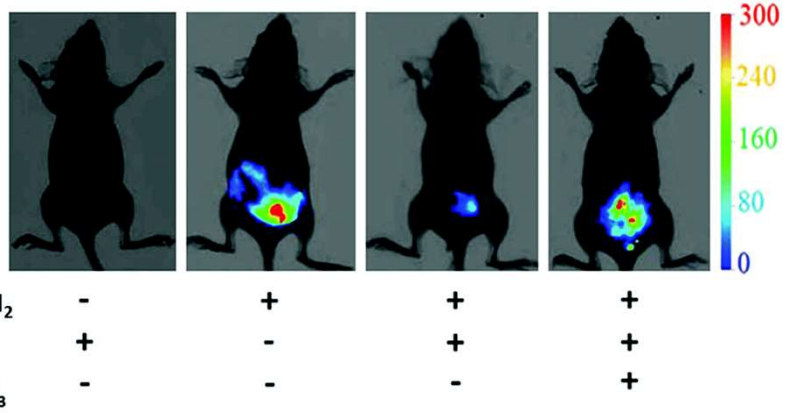
✓In vivo （Bruker In vivo Imaging System）

- Reversible

→To detect fluctuations



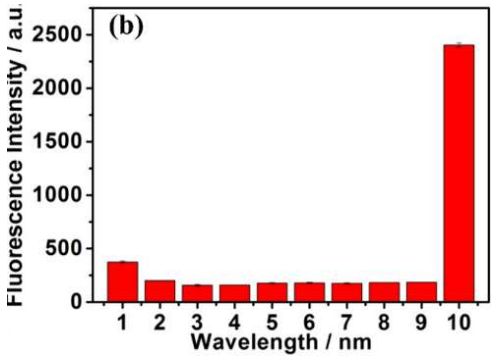




Fluorescence image of FA level changes in the peritoneal cavity of BALB/c mice. (a) Group a was injected in the i.p. cavity with FA (500 μM, 50 μL in 1 : 9 DMSO/saline, v/v) for **1 h** as the control. (b) Group b was injected in the i.p. cavity with BOD-NH$_2$ (50 μM, 50 μL in 1 : 9 DMSO/saline, v/v) for 1 h. (c) Group c was injected in the i.p. cavity with FA (500 μM, 50 μL in 1 : 9 DMSO/saline, v/v) for 1 h and then injected i.p. with BOD-NH$_2$ (50 μM, 50 μL in 1 : 9 DMSO/saline, v/v) for another 1 h. (d) Group d was pretreated as described in group c, but given $NaHSO_3$ (500 μM, 100 μL in 1 : 9 DMSO/saline, v/v) for one more hour.

(c) Time-dependent response cycles of BOD-NH$_2$ (5 μM) towards FA. FA was added at the reaction of 10 min in 10 mM HEPES buffer (pH 7.4, 0.5% Tw 80). 2 h later, the solution was treated with 500 μM $NaHSO_3$. When fluorescence returned to the starting levels, another 1 equiv. of FA was added to the mixture. The redox cycle was repeated 3 times. (d) Fluorescence responses of BOD-NH$_2$ (5 μM) to bio-relevant RCS, amino acids, and other relevant biological species. The bars represented relative responses at 515 nm of BOD-NH$_2$ to the analytes: 1, blank; 2, FA (500 μM); 3, FA (500 μM) then 250 μM $NaHSO_3$; 4, FA (500 μM) then 500 μM $NaHSO_3$; 5, acetaldehyde (50 μM); 6, methylglyoxal (50 μM); 7, glyoxal (50 μM); 8, benzaldehyde (50 μM); 9, pyridoxal (50 μM); 10, 4-nitro-benzaldehyde (50 μM); 11, sodium pyruvate (5 mM); 12, alanine (400 μM); 13, glycine (5 mM); 14, serine (5 mM); 15, arginine (5 mM); 16, cysteine (5 mM); 17, glutathione (5 mM); 18, glucose (1 mM); 19, hydrogen peroxide (100 μM); 20, hydrogen sulfide (100 μM); 21, methane acid (100 μM); and 22, dehydroascorbate (100 μM). All the above data were recorded in 10 mM HEPES buffer (10 mM, pH 7.4) at 37 ° C for 2 h. λ_{ex} = 495 nm, λ_{em} = 515 nm. The data are shown as mean (±s.d.) (n = 7).

AIE-FA



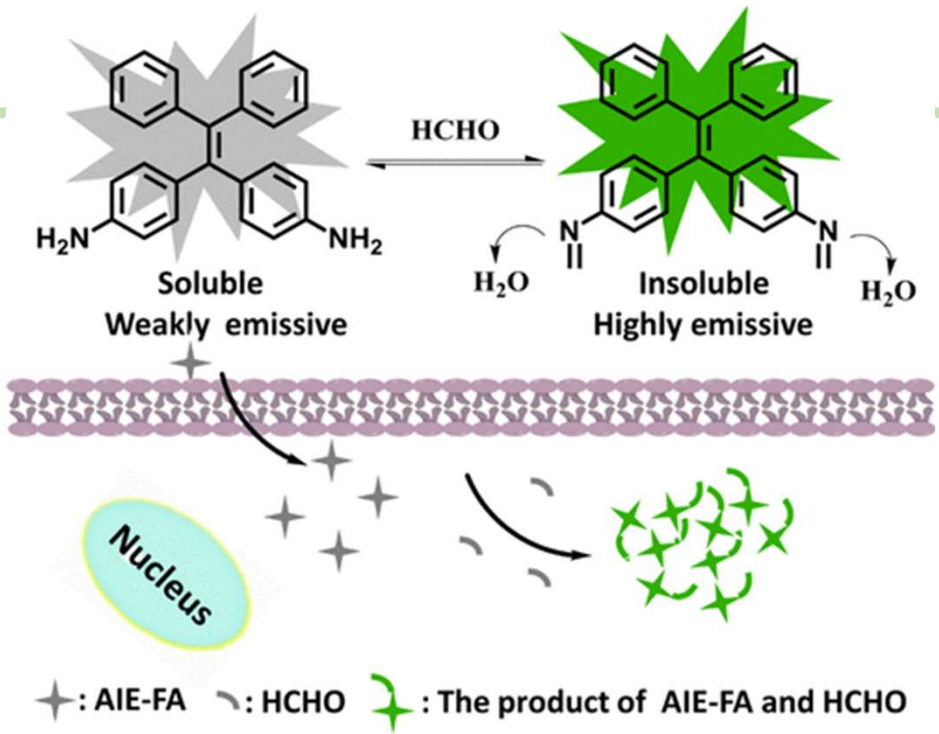
Selectivity of AIE-FA toward different relevant species in PBS, supplemented with 10% DMSO: (1) CH_3CHO (5 μM), (2) CHOCHO (5 μM), (3) CH_3COCHO (15 μM), (4) H_2O_2 (100 μM), (5) cysteine (1.0 mM), (6) glutathione (10 mM), (7) NaCl (100 mM), (8) KCl (50 mM), (9) $NaHSO_3$ (200 μM), and (10) FA (15 μM).

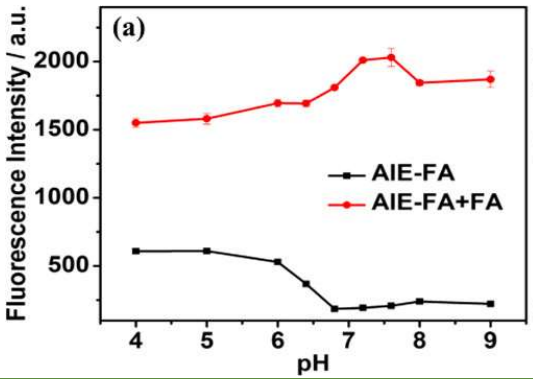
- Aggregation-Induced Emission

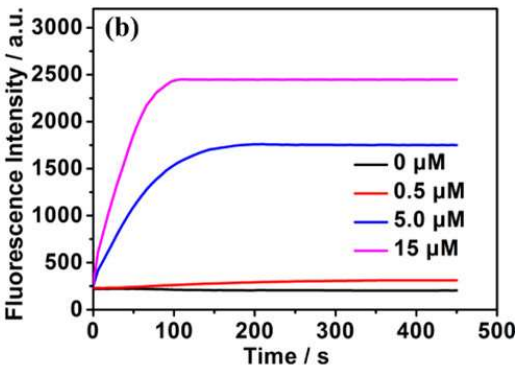
✓ Fast response

✓ Great selectivity

△PH sensitivity

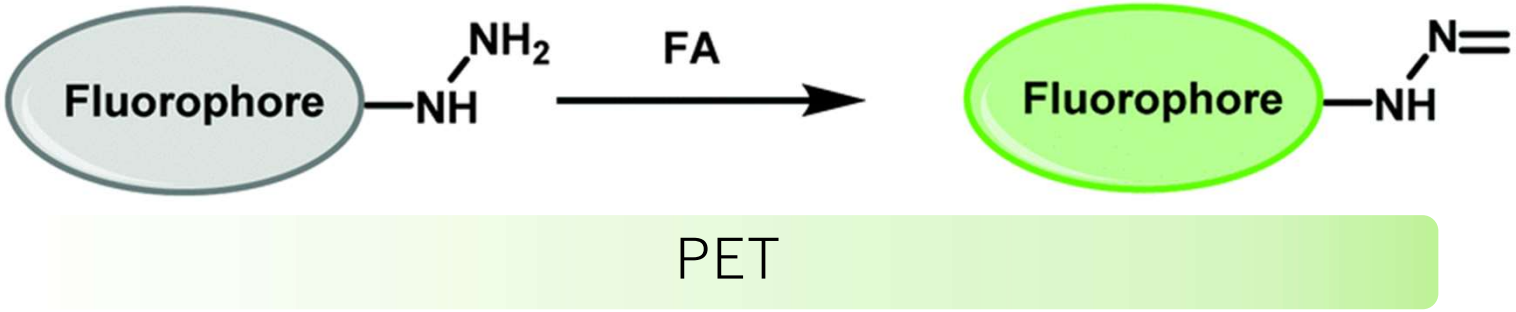






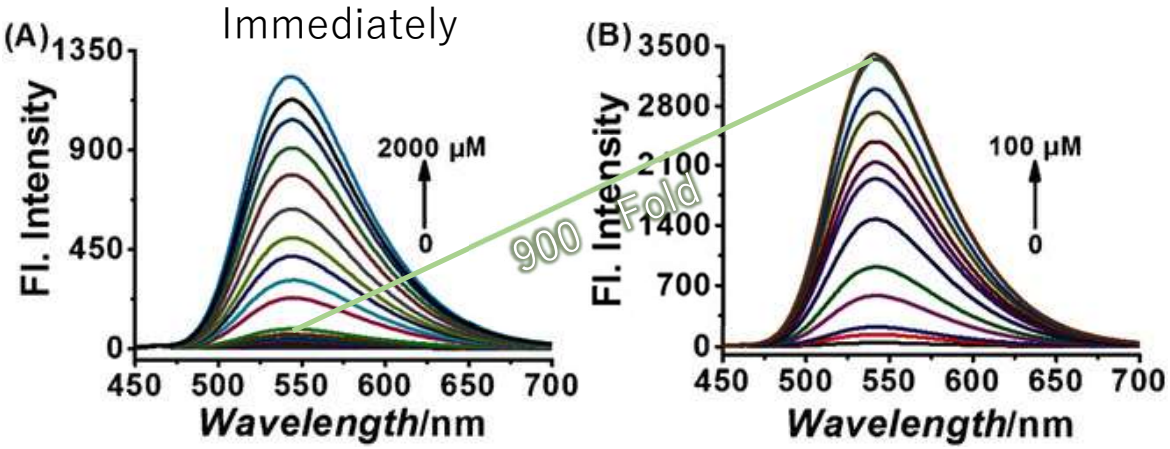
(a) Fluorescence intensity at 530 nm for AIE-FA (10 μM) with or without FA (10 μM) in buffers with different pH values (pH 4.0, 5.0, 6.0, 6.4, 6.8, 7.2, 7.6, 8.0, and 9.0), supplemented with 10% DMSO. (b) Real-time fluorescence responses of AIE-FA (10 μM) to different concentrations of FA (0, 0.5, 5.0, and 15 μM).

Hydrazine-based Probe

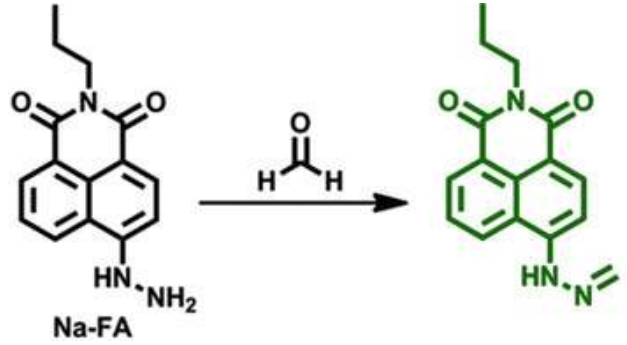


- Higher Stability

Na-FA



The fluorescence response of the probe **Na-FA** (**5 μM**) to FA at varied concentrations in PBS buffer (pH 7.4, 1 % DMSO). λ_{ex}=440 nm. A) Spectra were recorded **immediately** upon treatment of the probe with FA (0–2000 μM); B) Spectra were recorded after treatment of the probe with FA **(0–100 μM**) followed by a **30 min** incubation period.



- 2 photon

✓ Fast onset

✓ Large turn-on signal

✓ low detection limit

→For tracking of endogenous FA in living cells

Short Summary

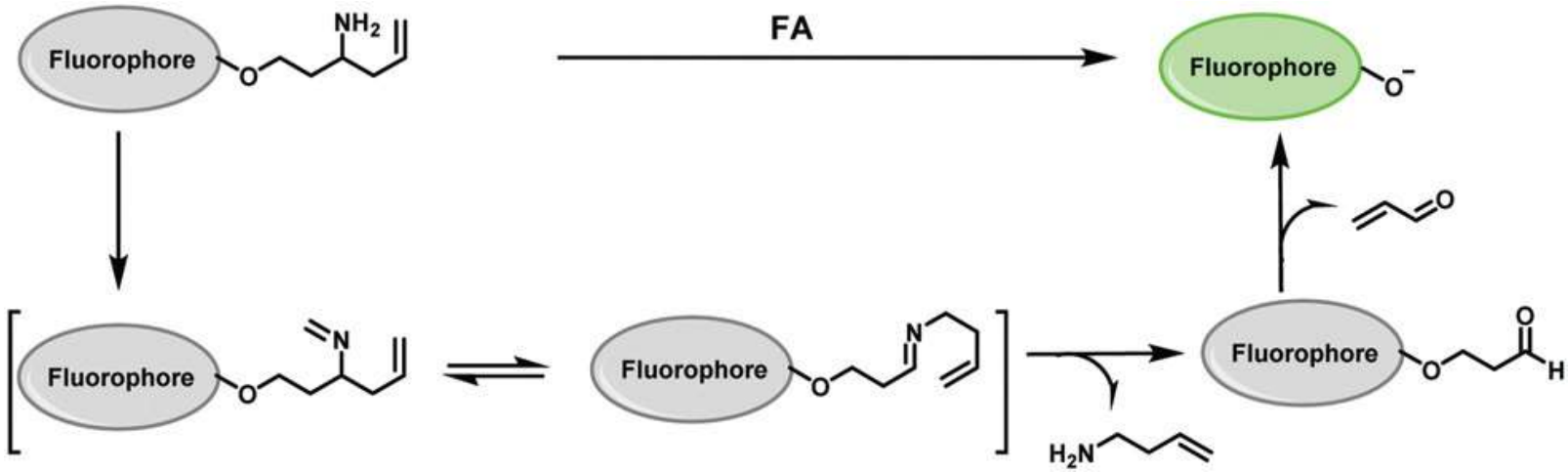
Formimine-based & Hydrazine-based Probe

✓High real-time resolution
✓High Spatial resolution
→Qualitive

△ Stability (Formimine-based Probe)
△ Low selectivity (Hydrazine-based Probe)
→~~Quantitative~~

×pH sensitivity

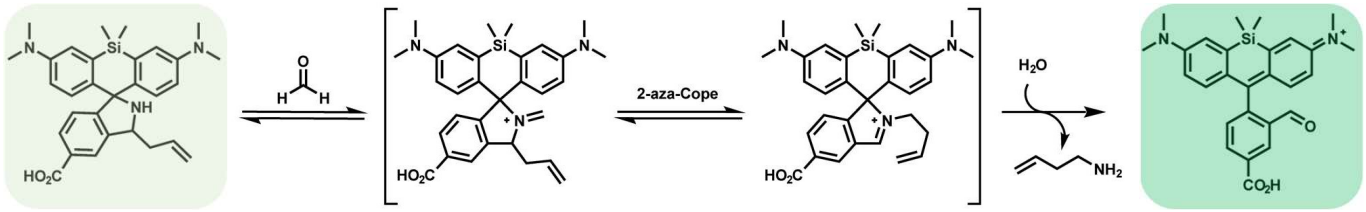
2-Aza-Cope Rearrangement-based probe



- Stability
- High Selectivity

→Quantification

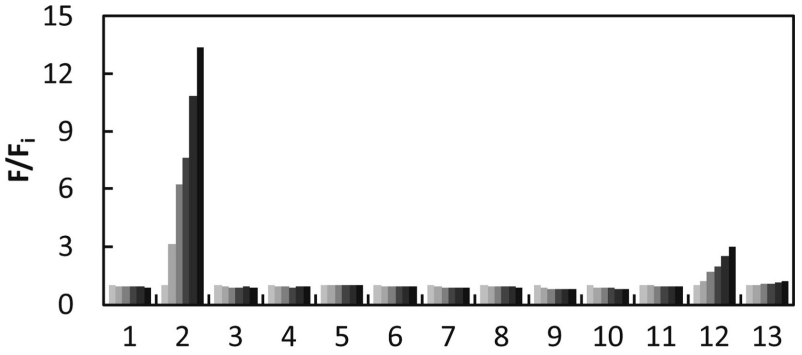
FAP-1



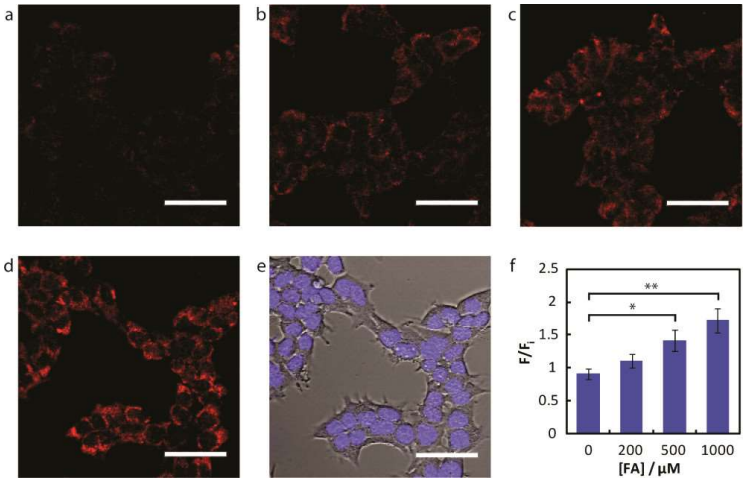
- Spirocyclization-based

✓ Good Selectivity

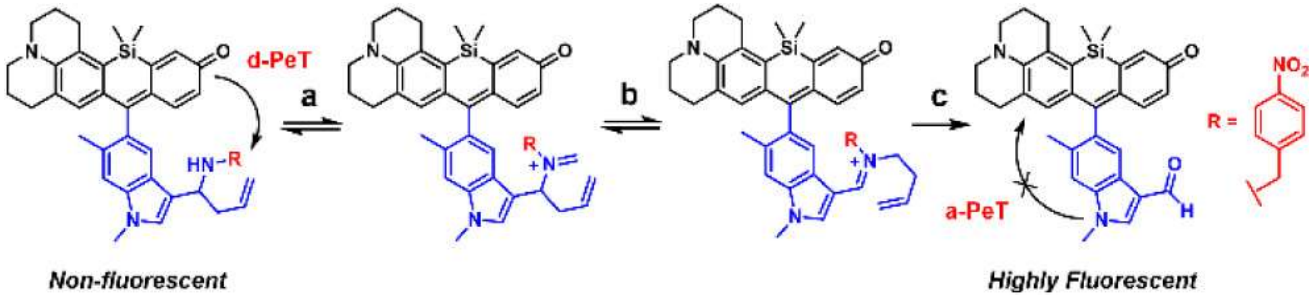
✓ In Cell

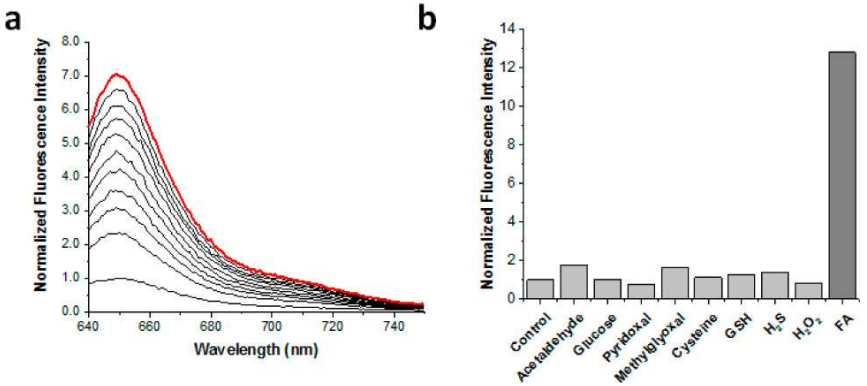


Legend: (1) PBS; (2) FA; (3) acetaldehyde; (4) 4-hydroxynonenal; (5) dehydroascorbate; (6) glucose, 1 mM; (7) glucosone; (8) oxaloacetate; (9) pyruvate; (10) H_2O_2; (11) glutathione, 5 mM; (12) methylglyoxal; (13) methylglyoxal, 10 μM.



FP1



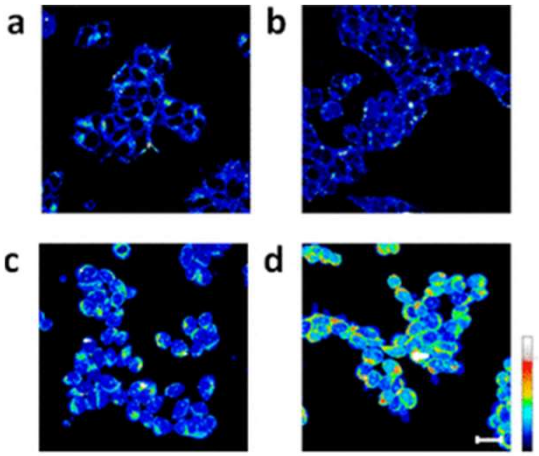


(a) Fluorescence response of 1 μM FP1 to **0.25 mM** FA in PBS buffer (pH 7.4) at 37 ° C. FP1 was excited at 633 nm, and the emission was collected between 640 and 750 nm**. Time points are 0, 15, 30, 45, 60, 75, 90, 105, 120, 150, and 180 min.** (b) Fluorescence response of 1 μM FP1 to biologically relevant aldehydes, reactive sulfur species, and hydrogen peroxide. Bars represent normalized fold changes in response to treatment with each analyte listed at 1 mM for **3 h**.

- 4-Nitrobenzyl for d-PeT

✓ High Sensitivity
✓ High Selectivity
✓ Stability
✓ In Cell

× Long incubation time

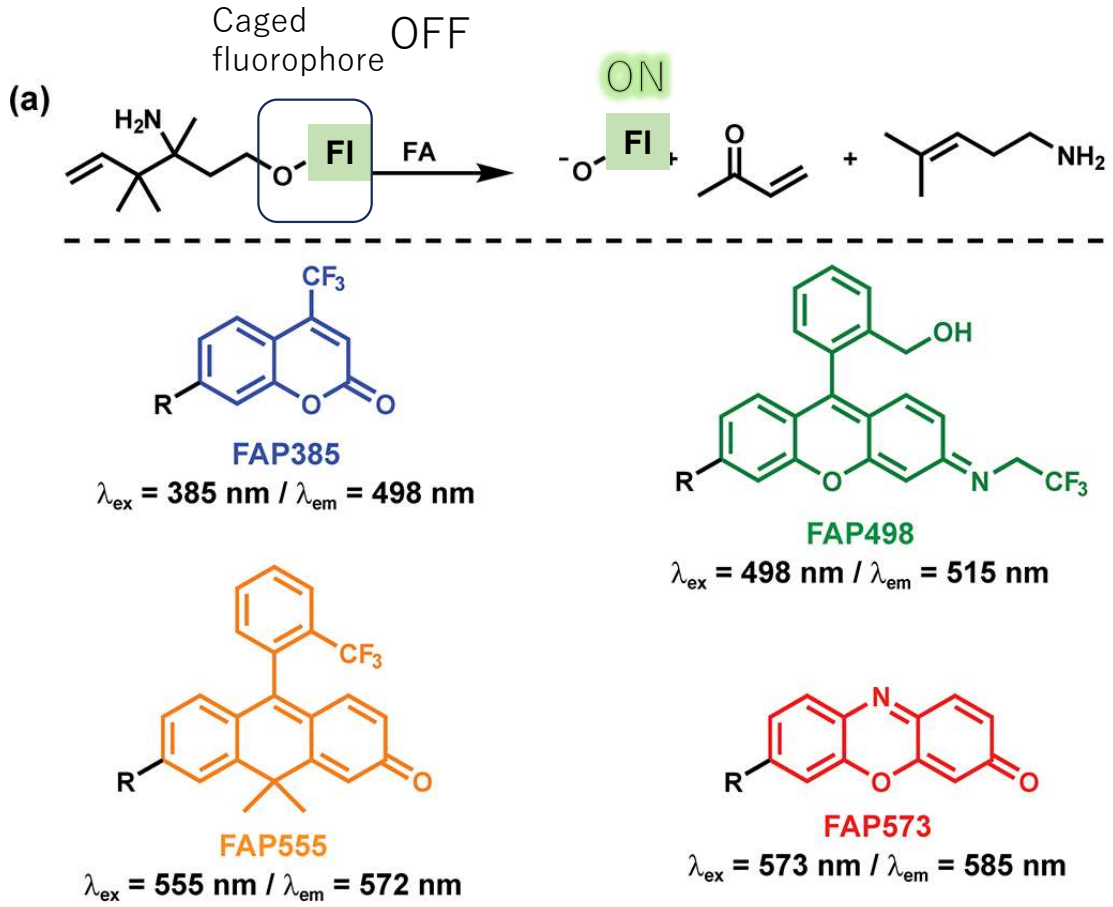


Confocal microscopy images acquired by irradiation of HEK293TN cells treated with (a) a DMEM vehicle control, (b) 1, (c) 2.5, and (d) 5 mM FA for **3 h** at 37 ° C with the 633 nm HeNe laser. Scale bar represents 20 μm.

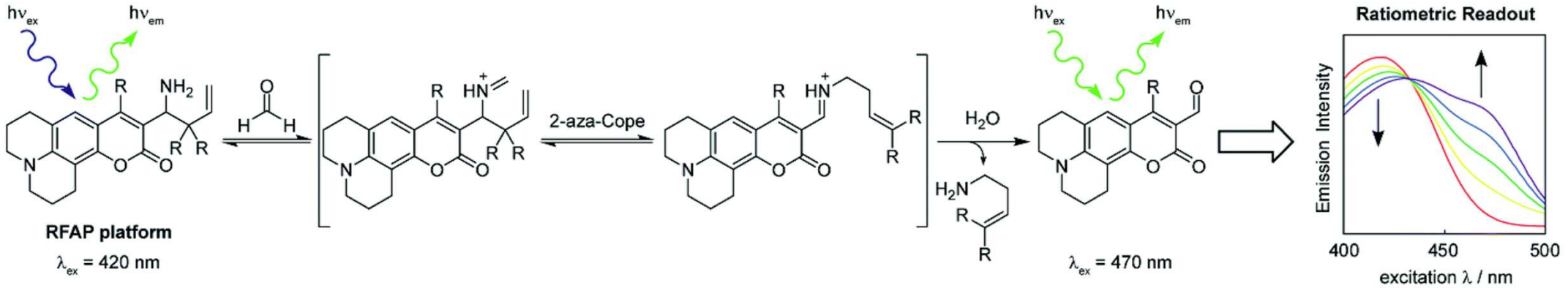
FA Trigger

- Caged
- Self immolative
- ✓ Selectivity

→Apply to multiple types of platforms

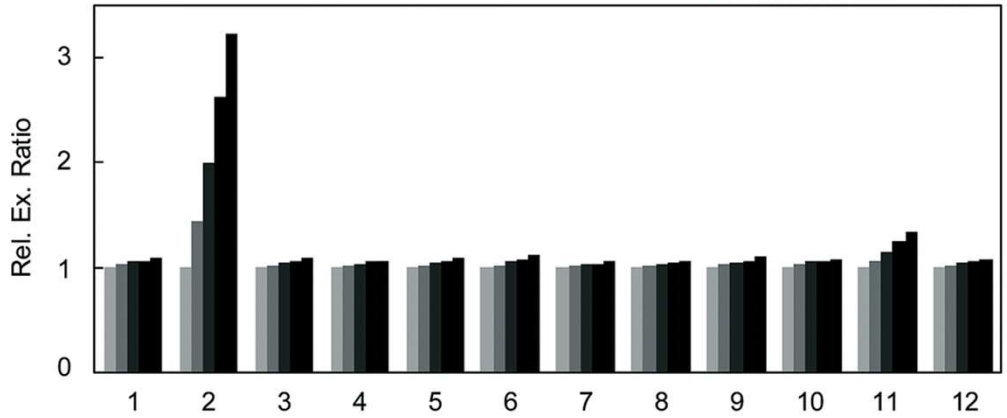


RFAP



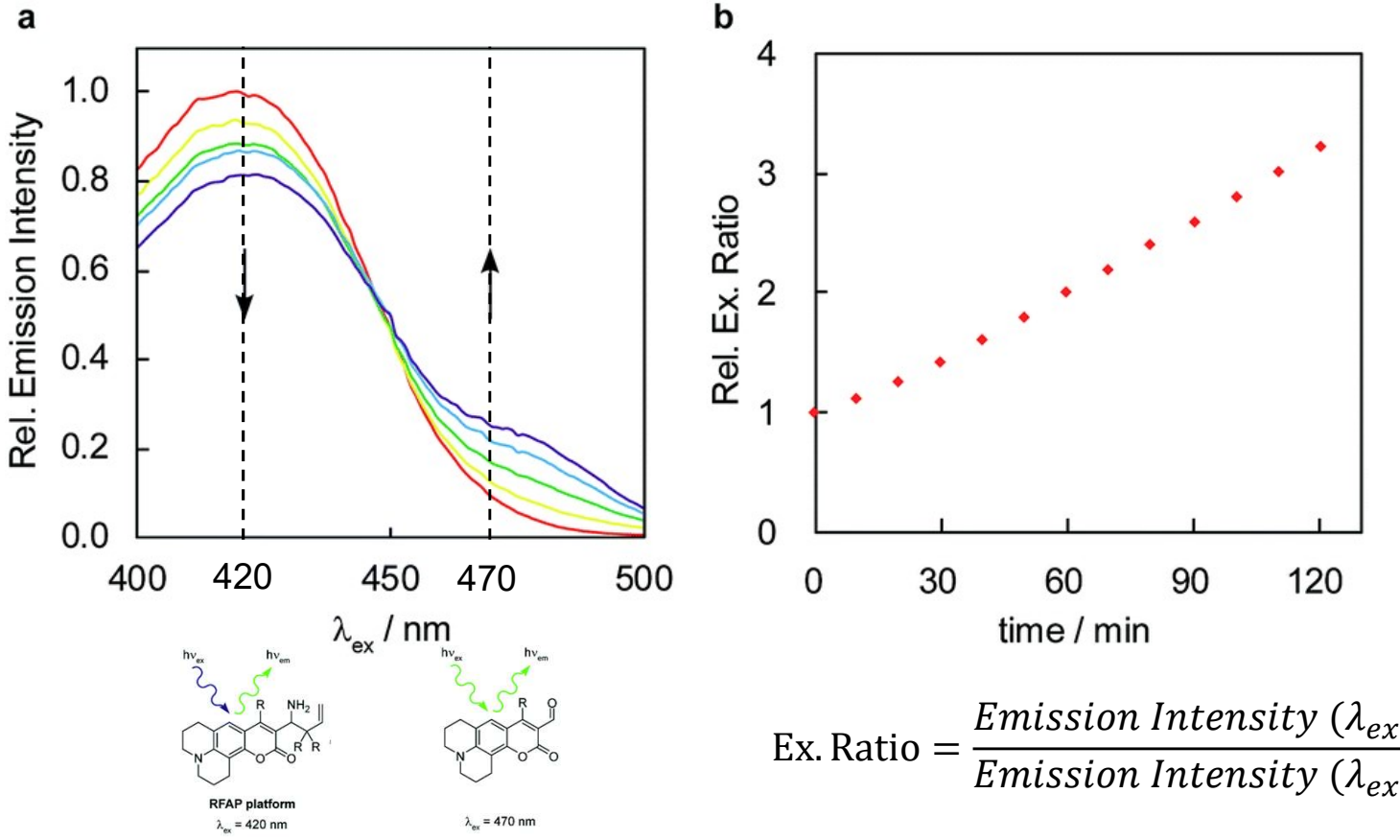
- Ratiometric imaging

✓ Selectivity
✓ Time-dependent
✓ Concentration-dependent



(1) PBS; (2) FA; (3) acetaldehyde; (4) pyruvate; (5) glucose, 1 mM; (6) 4-HNE; (7) dehydroascorbate; (8) oxaloacetate; (9) glucosone; (10) acrolein; (11) methylglyoxal; (12) methylglyoxal, 10 µM.

Ratiometric imaging

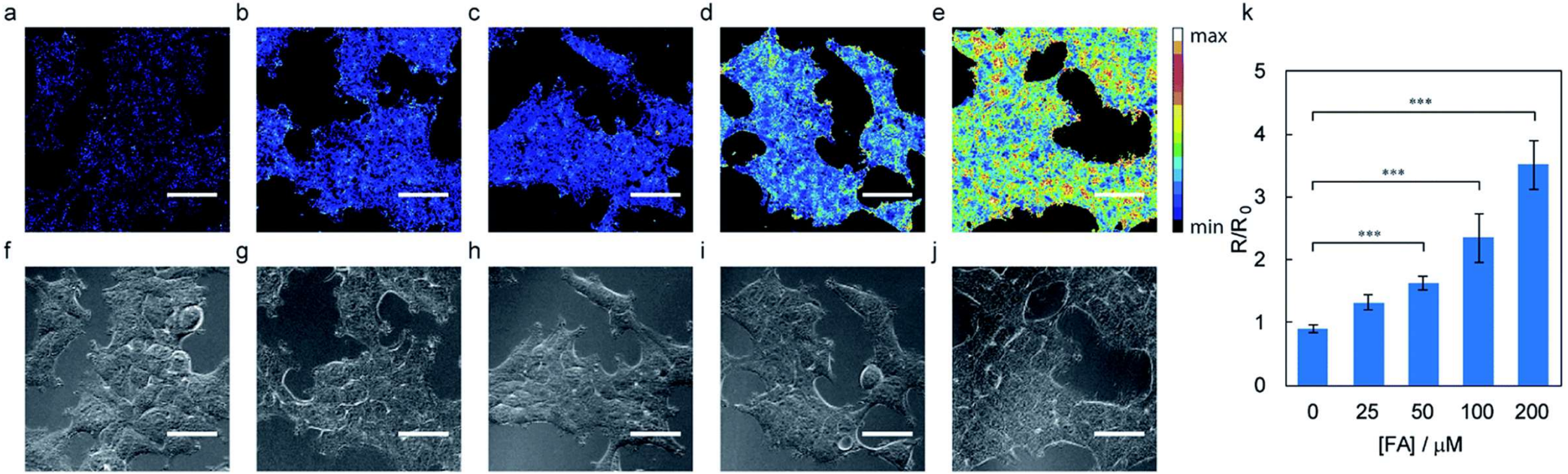


FA response and selectivity of RFAP-1. Data were acquired at 37 ° C in 20 mM PBS (pH 7.4). Excitation spectra were collected between 400 and 500 nm with emission monitored at λ_{em} = 510 nm. (a) Excitation ratiometric response of 10 µM RFAP-1 to 100 µM FA. Excitation spectra are shown at 0, 30, 60, 90, and 120 min (red, yellow, green, blue, and purple traces, respectively) after addition of FA. (b) Quantification of 470/420 nm excitation ratio over time.

$$\text{Ex. Ratio} = \frac{Emission\ Intensity\ (\lambda_{ex}470)}{Emission\ Intensity\ (\lambda_{ex}420)}$$

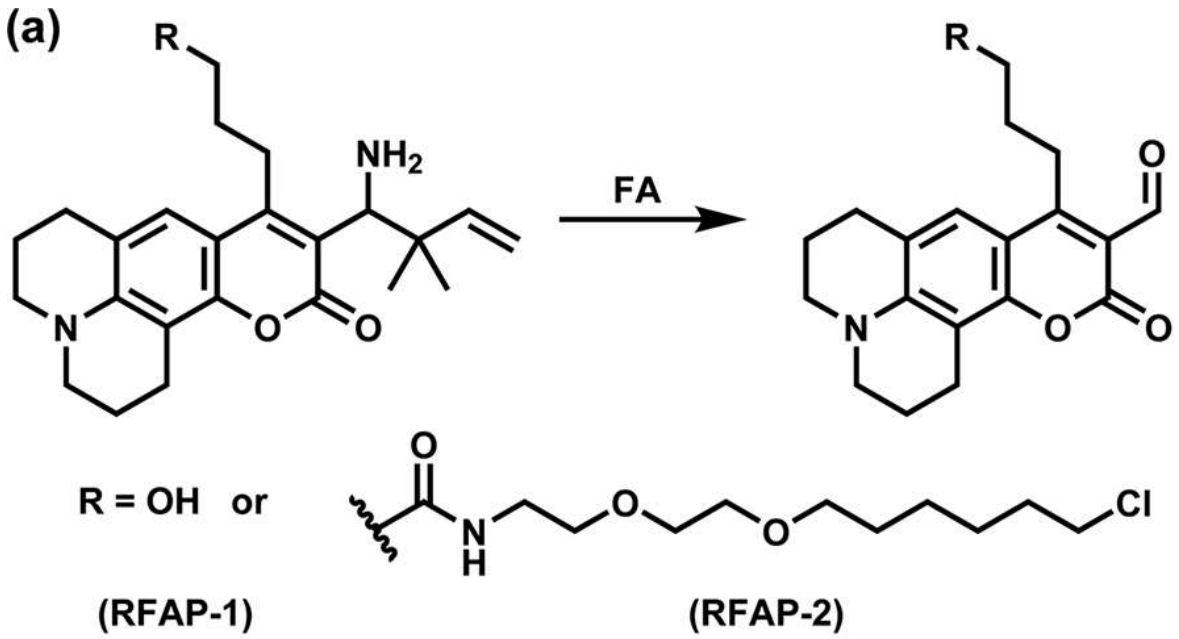
Ratiometric imaging

- Correlate with the concentration of FA



Representative ratiometric confocal microscopy images of FA detection in live HEK293T cells loaded with 10 μM RFAP-1. Images were taken **60 min** after the addition of (a) vehicle, (b) 25 μM FA, (c) 50 μM FA, (d) 100 μM FA, and (e) 200 μM FA. (f–j) Bright-field images of the cells in (a–e). Scale bar represents 40 μm in all images. (k) Mean 488/405 excitation ratios of the HEK293T cells treated with varying concentrations of FA for 60 min relative to the mean 488/405 excitation ratios before FA addition; error bars denote SEM, $n = 5$. ***$P < 0.001$.

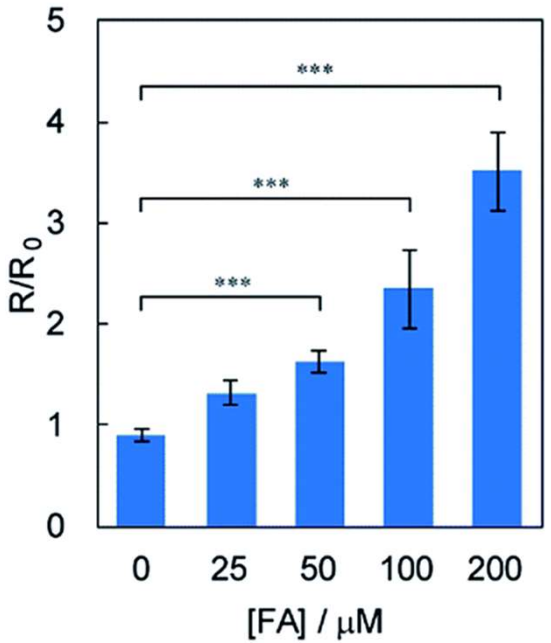
RFAP



Retention

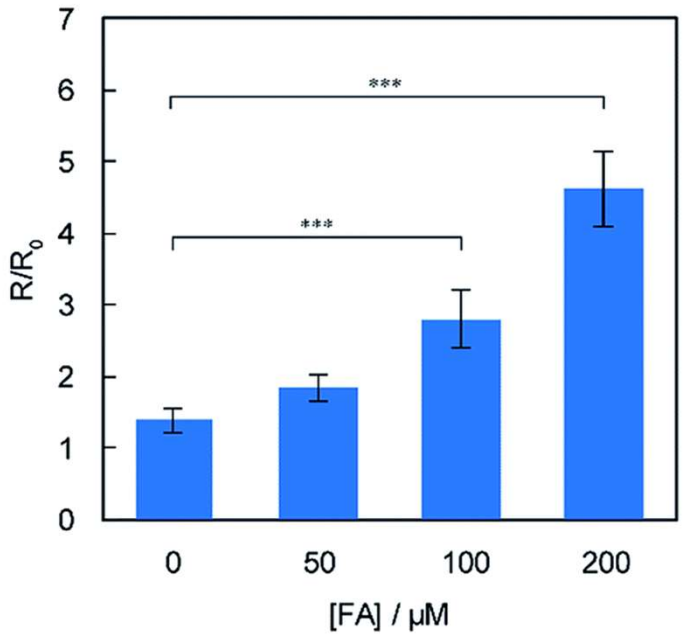
k

RFAP-1



i

RFAP-2

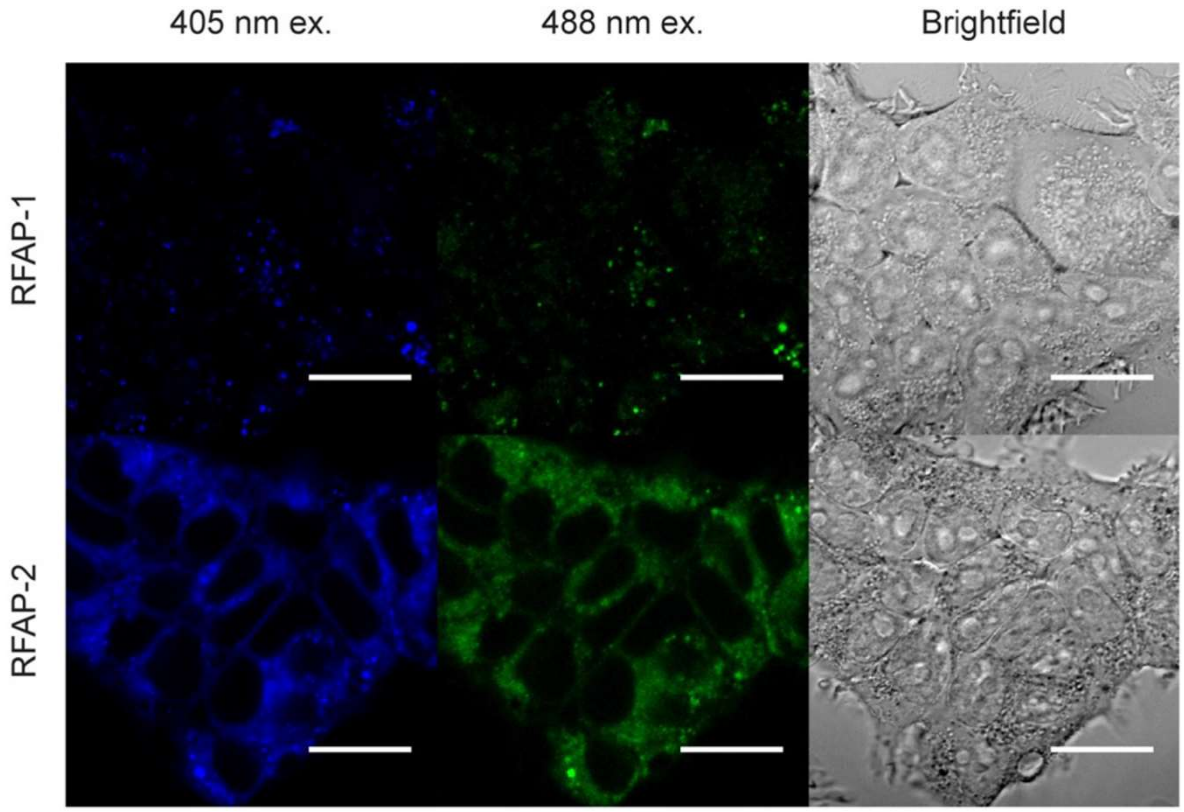


RFAP-2 showed better cellular retention.

(k) Mean 488/405 excitation ratios of the HEK293T cells treated with varying concentrations of FA with 10 μM RFAP-1 for **60 min** relative to the mean 488/405 excitation ratios before FA addition; error bars denote SEM, $n = 5$. ***$P < 0.001$

(i) Mean 488/405 excitation ratios of the HEK293T cells treated with varying concentrations of FA with 10 μM RFAP-2 for **60 min** relative to the mean 488/405 excitation ratios before FA addition; error bars denote SEM, $n = 4$. ***$P < 0.001$.

Staining

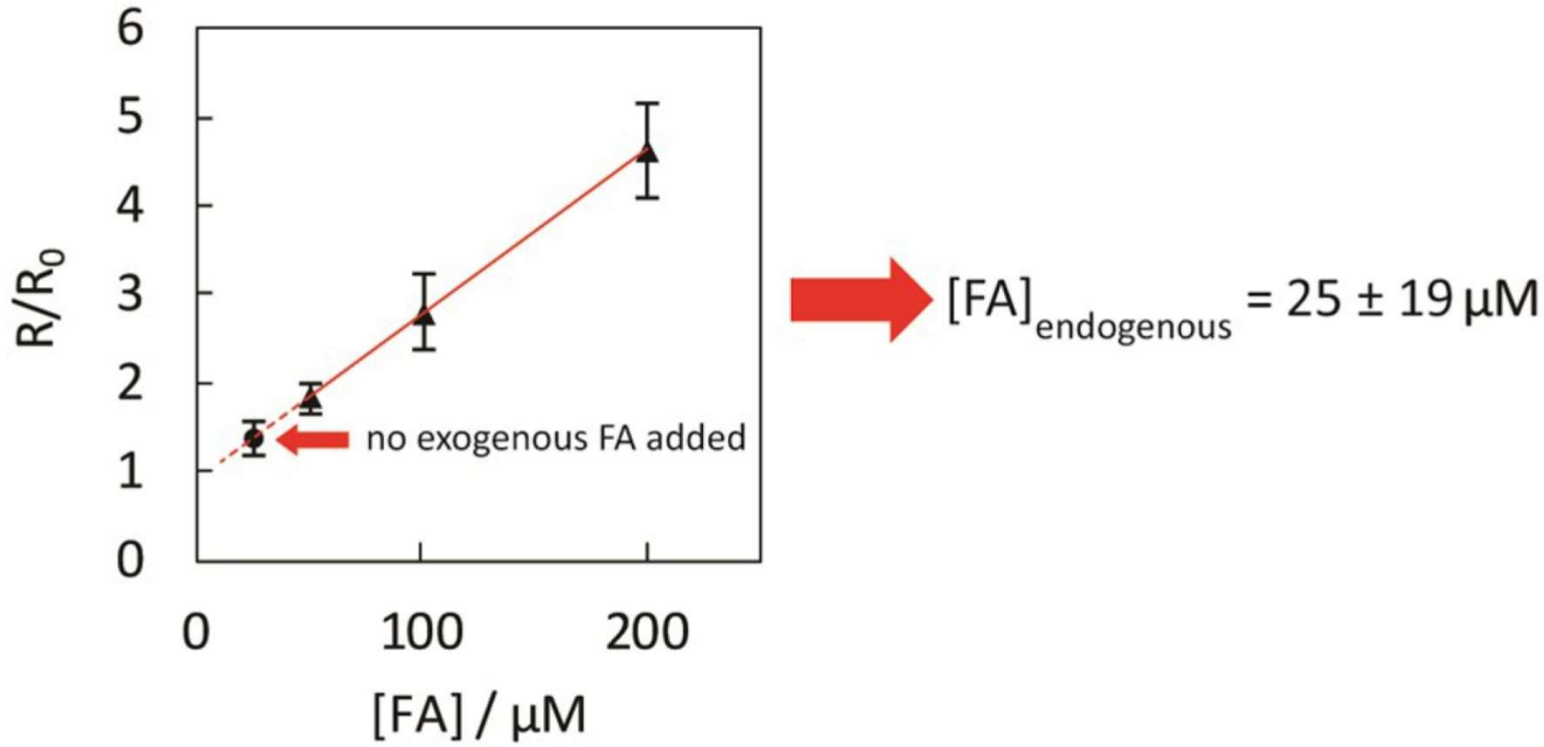


RFAP-1 displays punctate staining in HEK293T cells. HEK293T cells were treated with 10 μM RFAP-1 or RFAP-2 in BSS for **30 min** at 37 ° C, washed with fresh BSS, then imaged. Scale bar represents 20 μm.

RFAP-2 showed better cellular staining.

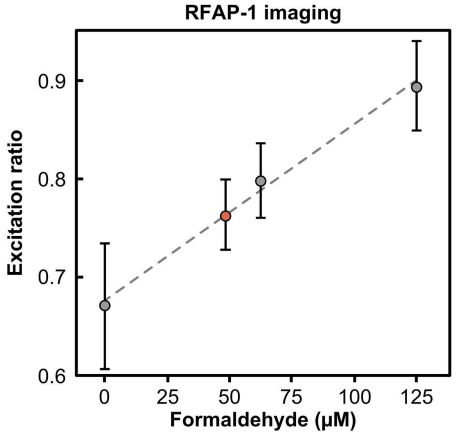
Brewer, T. F.; Chang, C. J. *et al. Chemi. Sci.* **2017**, *8*, 4073–4081

Using RFAP-2 to detect cells FA level



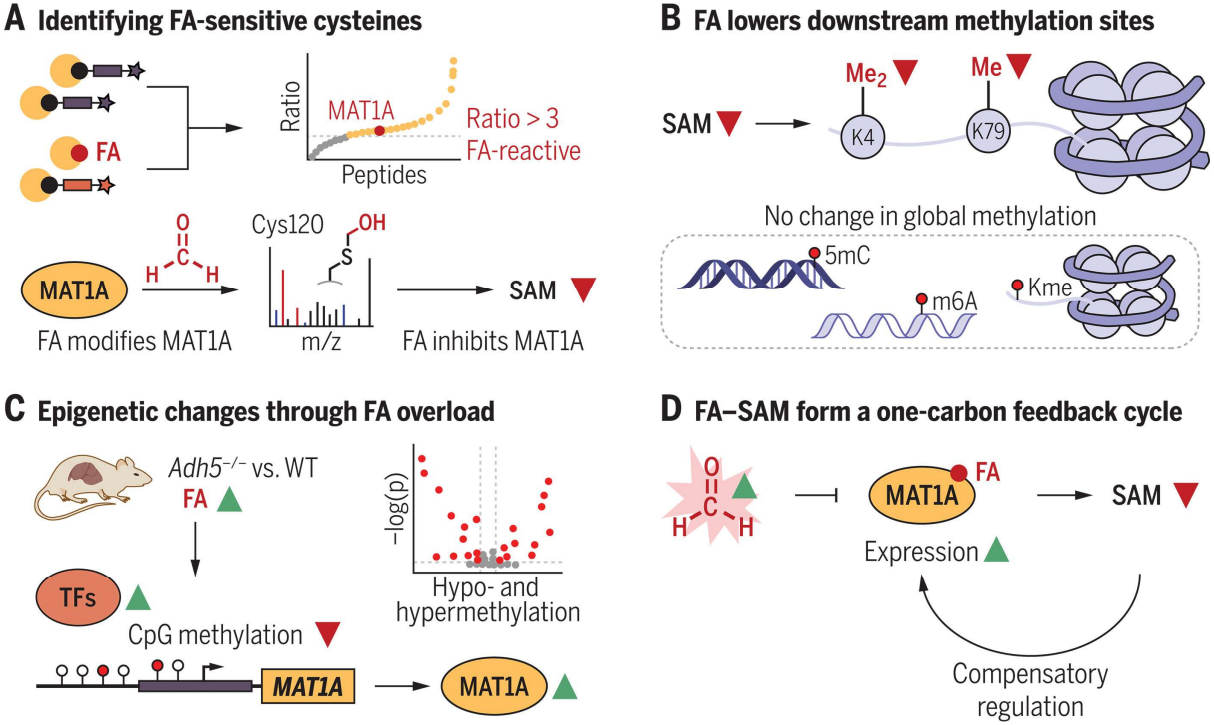
Calibration curve for [FA] in cells using RFAP-2. Intracellular [FA] was assumed to be equivalent to exogenous [FA] (except in case of no exogenous FA) to construct calibration curve. Leastsquares fit was extrapolated to observed R/R0 for [FA]exogenous = 0 μM to give predicted [FA]endogenous = 25 ± 19 μM, where ± 19 μM is the 95% confidence interval.

Application



Standard addition curve of FA in HepG2 by RFAP-1 fluorescent probe. Error bars represent the SD (n = 4) of technical cell replicates for each concentration.

Con.[FA]=49±10μM



- FA regulates S-adenosylmethionine biosynthesis by react with MAT1A.

Short Summary

2-Aza-Cope Rearrangement-based Probe

✓Good Stability

✓Good Selectivity

→Quantitative

×Long time to detect

Contents

- Introduction
- Fluorescent Formaldehyde Probe
  - Formimine-based
  - Hydrazine-based
  - 2-aza-Cope Rearrangement-based
- Summary

Summary

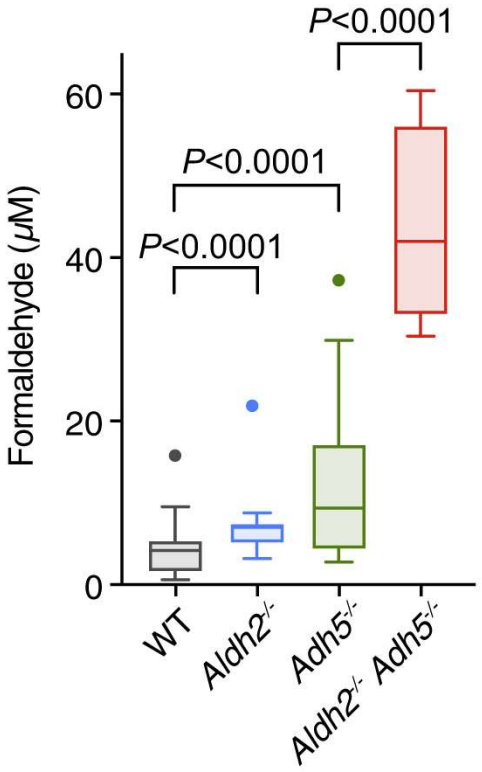
	Formimine	Hydrazine	2-aza-Cope Rearrangement
Spatial	☆☆☆	☆☆☆	☆☆☆
Time	☆☆☆	☆☆☆	☆
Sensitivity	☆☆☆	☆☆☆	☆☆☆
Selectivity	☆☆	☆☆	☆☆☆
Stability	☆	☆☆	☆☆☆
Con. dependent	☆	☆	☆☆☆
Application	Qualitative	Qualitative	Quantitative

Thanks for your attention, and

Please feel free to ask

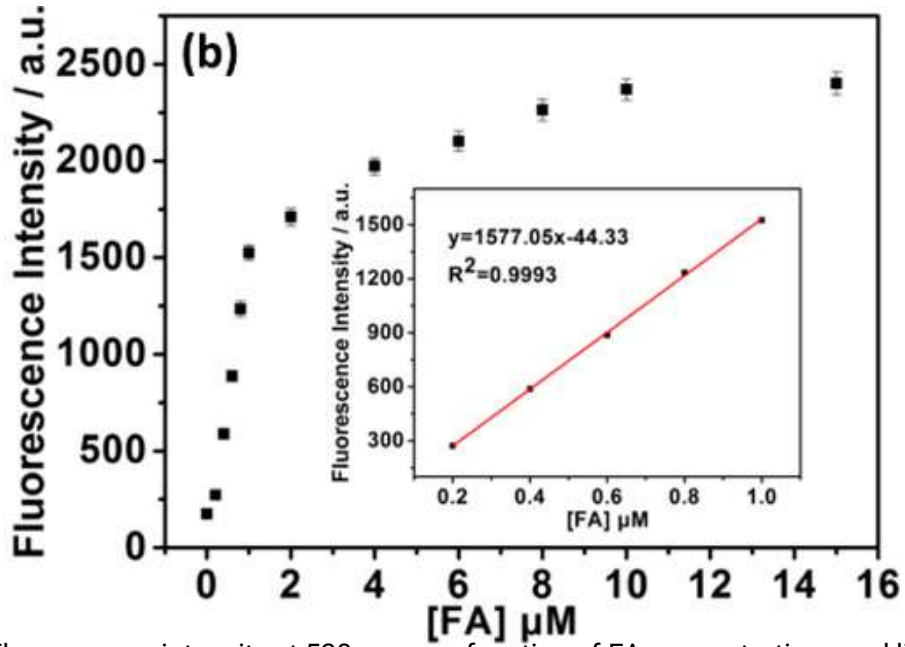
Appendix

FA is mainly decomposed by ALDH2 & ADH5



Serum levels of formaldehyde (n = 43, 20, 51, 4, left to right). Boxes with lines indicate quartiles and median, and Tukey whiskers extend to 1.5 interquartile ranges. Two-tailed Mann-Whitney *U* test.

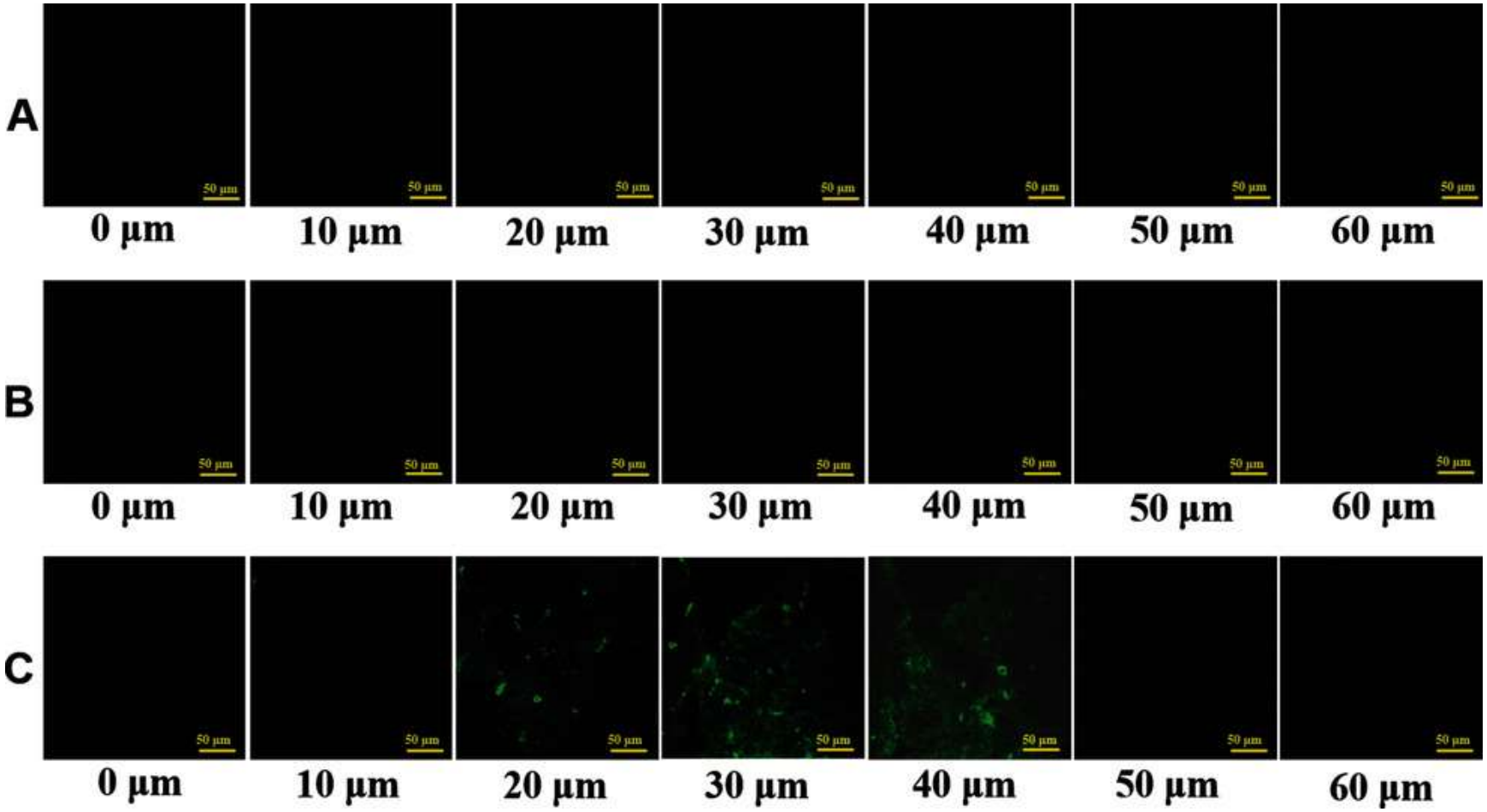
Detection limit of AIE-FA



Fluorescence intensity at 530 nm as a function of FA concentrations and linear fit between the fluorescence intensity and the concentration of FA (inset).

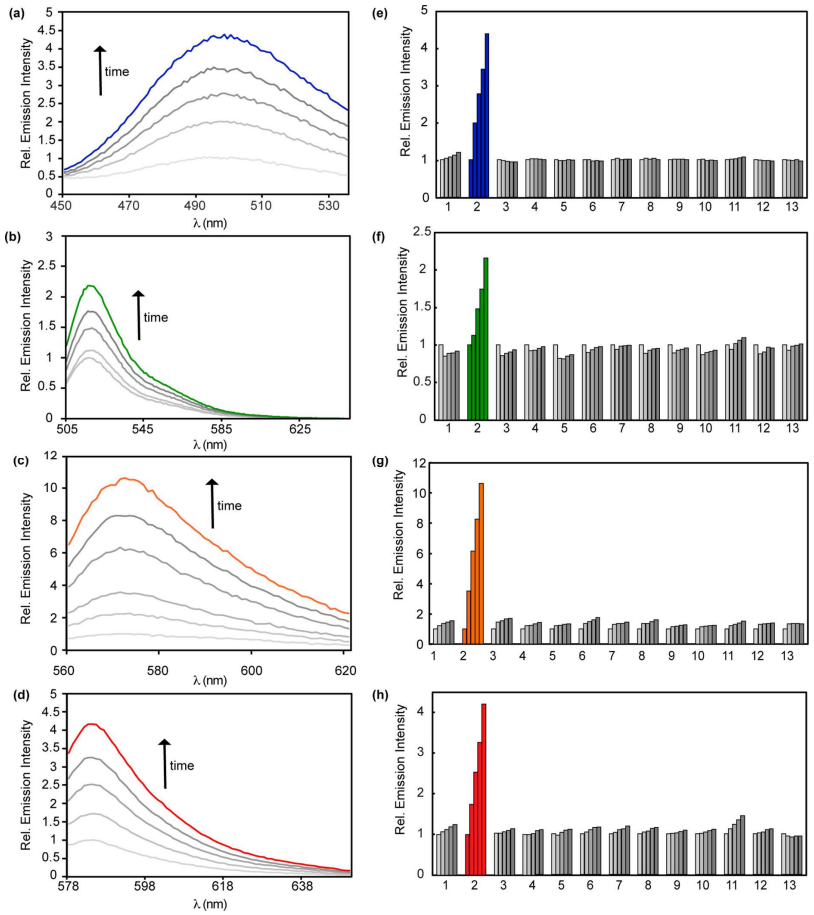
”FA concentration was obtained in the range of 100 nM to 1 μM with a detection limit estimated to be 40 nM.”

Na-FA liver tissue



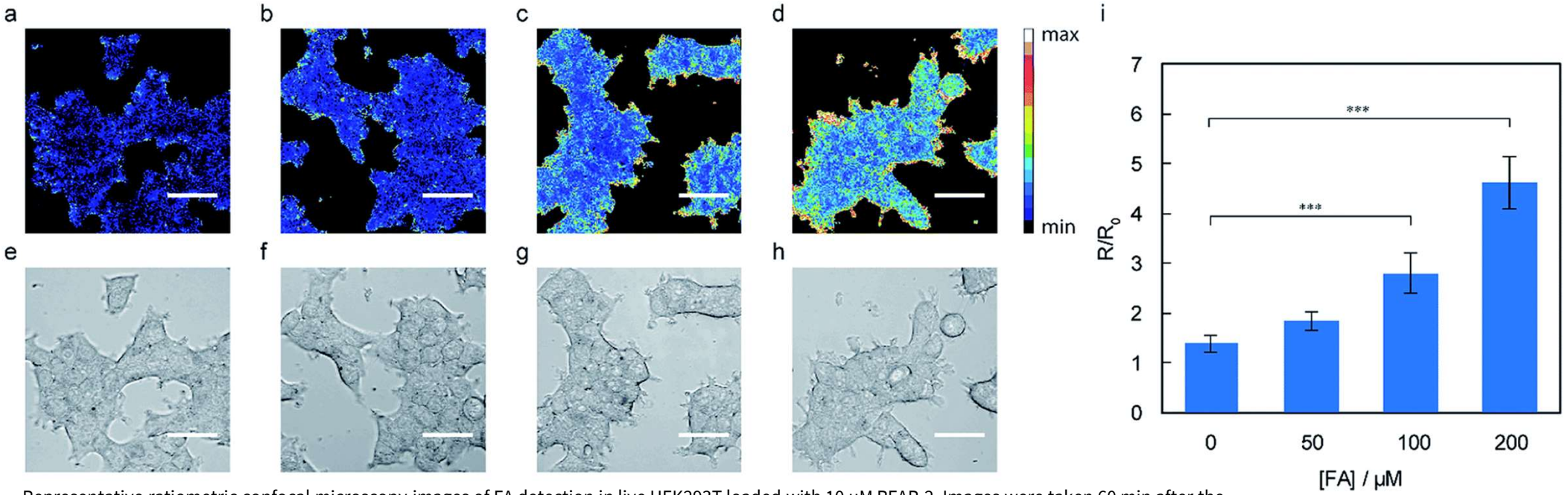
Two-photon fluorescence imaging of endogenous FA in liver slides. A) Fluorescence images of liver slides. B) Fluorescence images of liver slides incubated with the inhibitor $NaHSO_3$ for 30 min, and then with the probe (10 μM) for another 1 h. C) Fluorescence images of liver slides incubated with probe (10 μM) for 1 h. Excitation was at 880 nm by femtosecond laser, and the emission collection was from 500–550 nm. Scale bar: 50 μm. Labels from 0–60 μm indicate scanning depths of the tissue slices.

FA Trigger's selectivity



Fluorescence responses and selectivities of FA probes. (a–c) Fluorescence responses of 10 μM (a) **FAP385**, (b) **FAP498**, (c) **FAP555**, or (d) **FAP573** to 100 μM FA. Data were acquired in 20 mM PBS (pH 7.4) at 37 ° C. Emission was collected between (a) 450–535 nm (λ_{ex} = 385 nm), (b) 505–645 nm (λ_{ex} = 498 nm), (c) 560–625 nm (λ_{ex} = 555 nm) or (d) 578–650 nm (λ_{ex} = 573 nm). Lines represent time points taken at **0 (lightest gray), 30 (light gray), 60 (gray), 90 (dark gray), and 120 min (colored)** after addition of 100 μM FA. (e–h) Fluorescence responses of 10 μM probe to RCS or relevant biological analyte. Bars represent emission intensity responses to 100 μM analyte unless otherwise stated for 0 (lightest gray), 30 (light gray), 60 (gray), 90 (dark gray), and 120 (darkest gray) min, except FA, which is shown in colored bars. Analytes were prepared as stated in the Selectivity Tests section of the SI. Legend: (1) PBS, (2) FA, (3) acetaldehyde, (4) glucose (1 mM), (5) 4-hydroxynonenal, (6) dehydroascorbate, (7) glucosone, (8) pyruvate, (9) oxaloacetate, (10) acrolein, (11) methylglyoxal, (12) H_2O_2, (13) glutathione (5 mM).

RFAP-2



Representative ratiometric confocal microscopy images of FA detection in live HEK293T loaded with 10 μM RFAP-2. Images were taken 60 min after the addition of (a) vehicle, (b) 50 μM FA, (c) 100 μM, and (d) 200 μM FA. (e–h) Bright-field images of the cells in (a–d). Scale bar represents 40 μm in all images. (k) Mean 488/405 excitation ratios of the HEK293T cells treated with varying concentrations of FA for 60 min relative to the mean 488/405 excitation ratios before FA addition; error bars denote SEM, $n = 4$. ***$P < 0.001$.

SAM

- S-adenosylmethionine
- Key methyl donor