Literature Report

Reporter: 许宁

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Research Article

Designer Fluorescent Adenines Enable Real-Time Monitoring of MUTYH Activity

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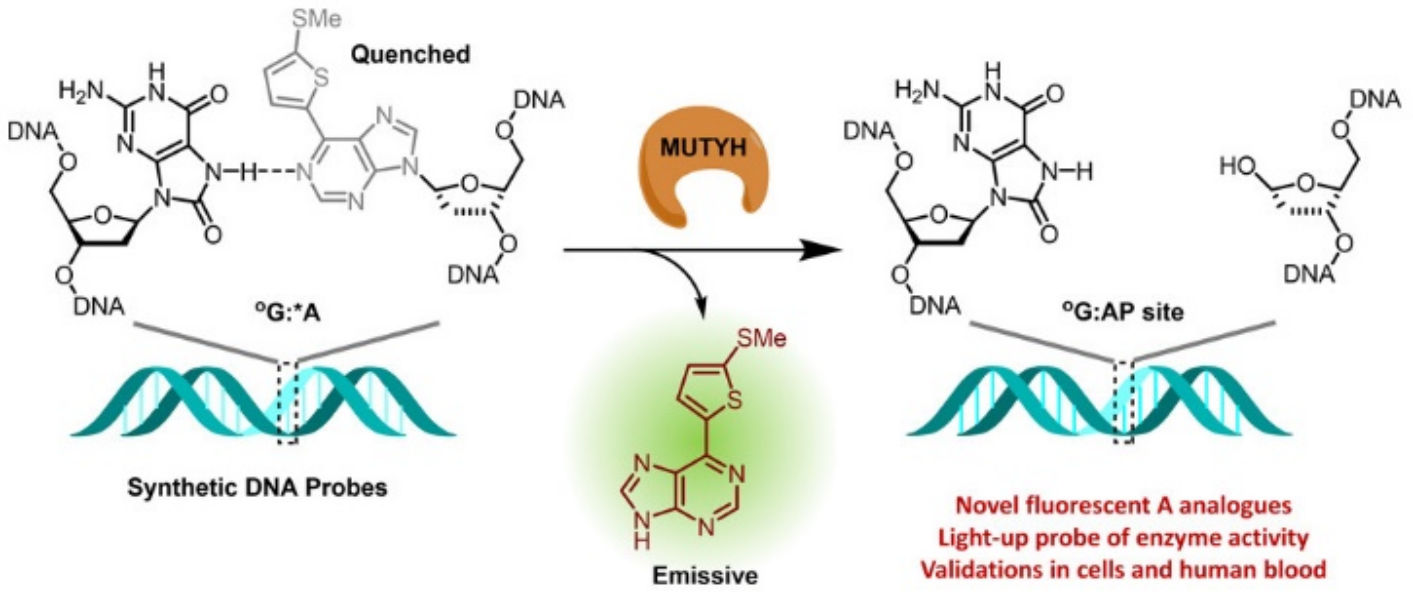

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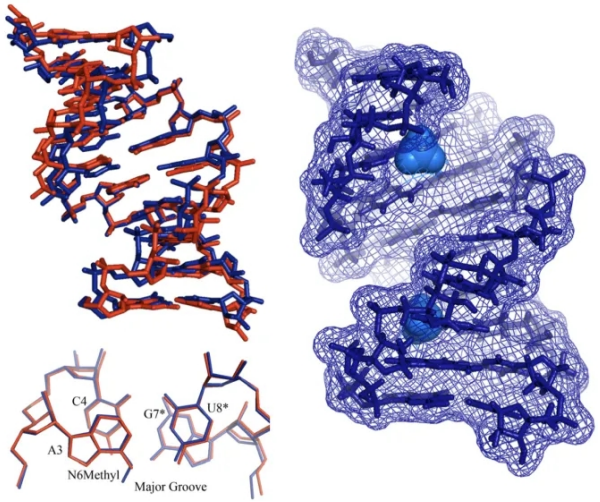
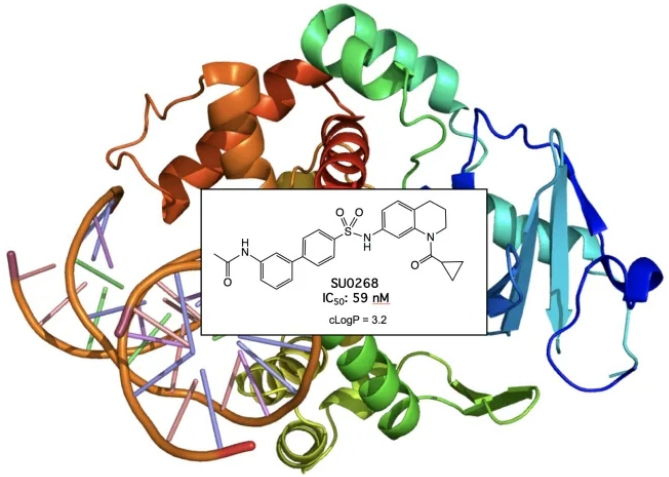
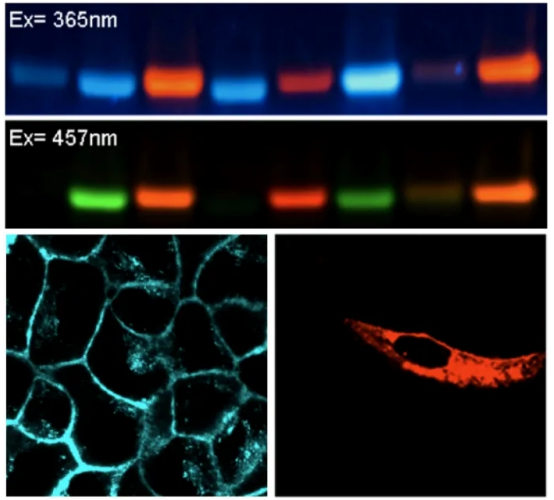
At Stanford he is a member of the Bio-X program, the Biophysics program, the Stanford Cancer Institute, and is a Fellow of ChEM-H.



RNA Chemical Biology



DNA Repair and Cancer



Imaging and Sensing

Oxidative DNA damage

氧化DNA损伤：由内源性和外源性活性氧（ROS）攻击引起的氧化性DNA损伤。由于鸟嘌呤具有最低的氧化还原电位，是最易被氧化的DNA碱基，产生**8-氧代-7,8-二氢鸟嘌呤（8-oxoG）**和2–6-二氨基-4-羟基-5-甲酰胺嘧啶（FapyG）等产物。

OGG1 ：8-氧鸟嘌呤糖基化酶，是一种在人类中由OGG1基因编码的DNA糖基化酶，它既可以切割诱变病变的糖苷键，又可以在DNA主链中引起链断裂。

MTH1：2-羟基-dATP二磷酸酶，是一种在人类中由NUDT1基因编码的酶，在DNA修复过程中，它可以水解氧化嘌呤并阻止它们添加到DNA链上。

MUTYH：mutY DNA糖基化酶，是一种单功能的糖基化酶，它参与氧化DNA损伤修复，它在腺嘌呤与鸟嘌呤，胞嘧啶或8-氧代-7,8-二氢鸟嘌呤（氧化性DNA损伤的常见形式）错配的部位，从DNA主链上切除腺嘌呤碱基。

Free Radical Biol. Med. 2017, 179−201.

MUTYH具有重要的生物学和潜在的治疗意义

OPEN ACCESS Freely available online

PLoS one

The *Mutyh* Base Excision Repair Gene Influences the Inflammatory Response in a Mouse Model of Ulcerative Colitis

减少胰腺癌细胞生长并增加化疗敏感性

Ida Casorelli[1*], Tania Pannellini[2], Gabriele D
Iavarone[1], Alessandro Giuliani[1], Alessia Butera
Bignami[1*]

www.impactjournals.com/oncotarget/ Oncotarget, 2017, Vol. 8, (No. 6), pp: 9216-9229

Research Paper

减少炎症反应

MutY-Homolog (MYH) inhibition reduces pancreatic cancer cell growth and increases chemosensitivity

ana[1], Amanda Mawson[1], Joshua McCarroll[2,3], Andrea
ber Johns[5], David Goldstein[1], Phoebe Phillips[1,3]

THE JOURNAL OF Pathology
A Journal of The Pathological Society
Understanding Disease

Original Paper

Reduced expression of MUTYH with suppressive activity against mutations caused by 8-hydroxyguanine is a novel predictor of a poor prognosis in human gastric cancer

Kazuya Shinmura, Masanori Goto, Masaya Suzuki, Hong Tao, Hidetaka Yamada, Hisaki Igarashi, Shun Matsuura, Matsuyoshi Maeda, Hiroyuki Konno, Tomonari Matsuda, Haruhiko Sugimura

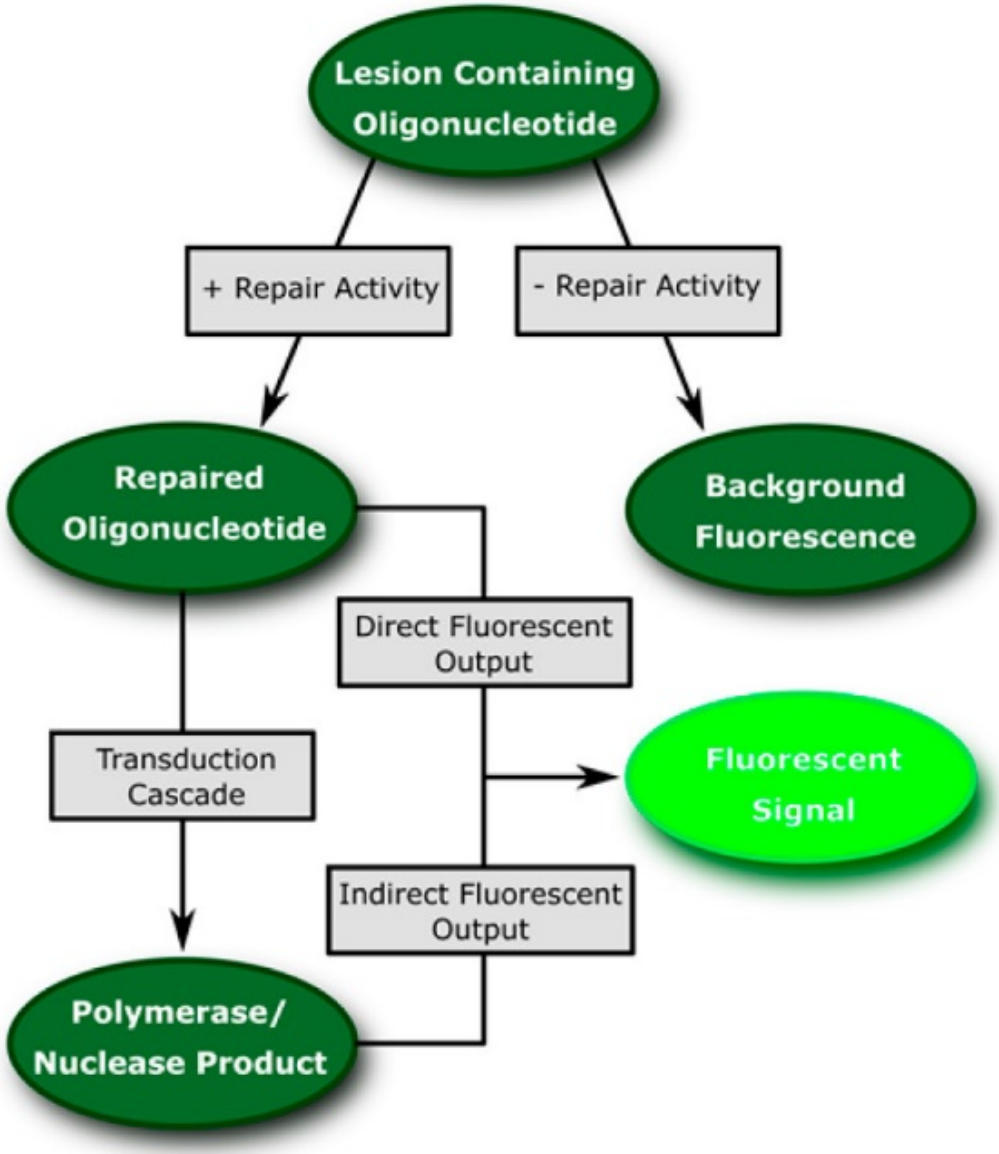
First published: 09 June 2011 | https://doi.org/10.1002/path.2953 | Citations: 36

临床有用的生物标志物

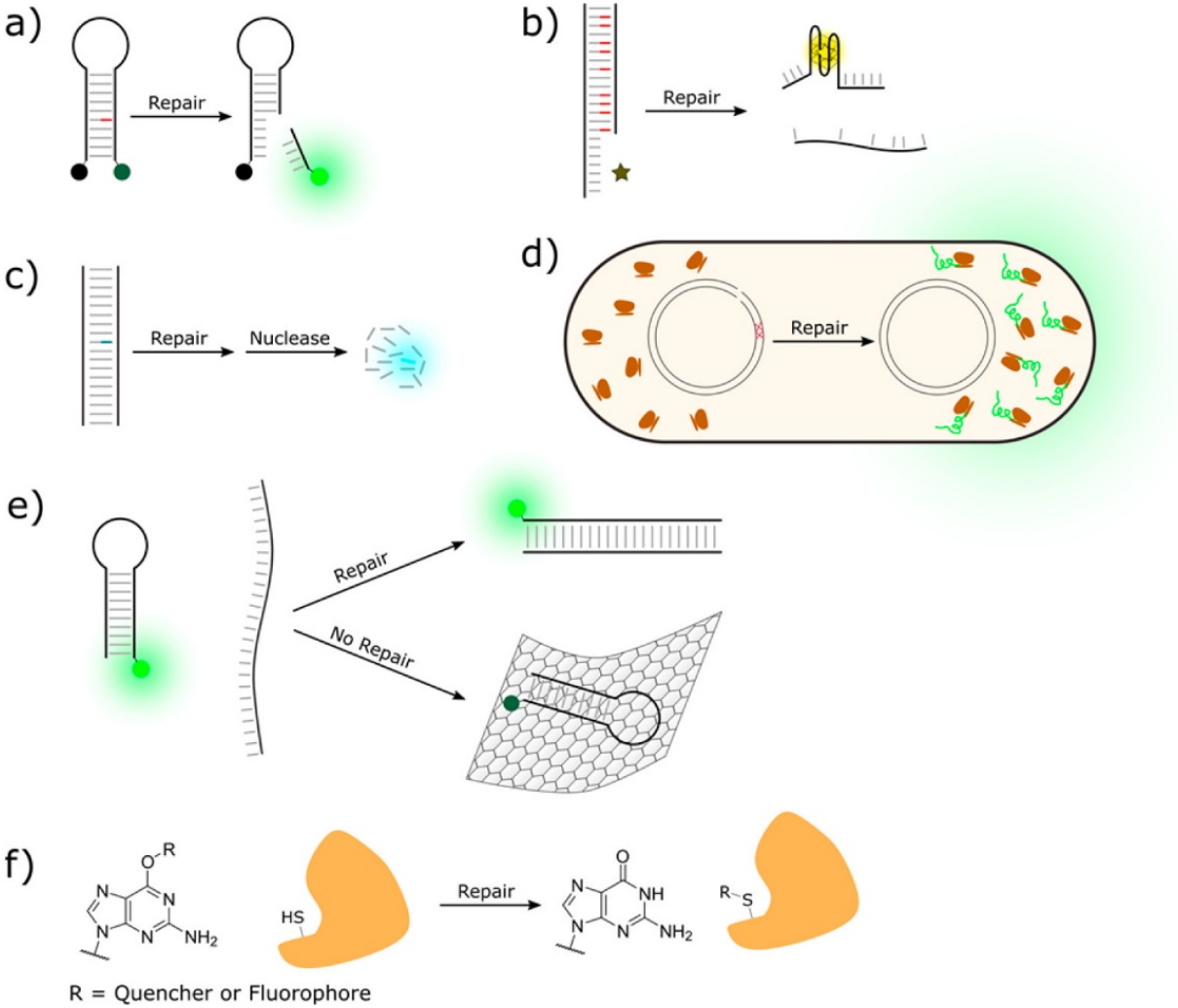
目前，测量MUTYH活性的主要方法是电泳和辐射分析。

缺乏实时监测MUTYH酶活性水平的方法

Fluorescent Probes of DNA Repair

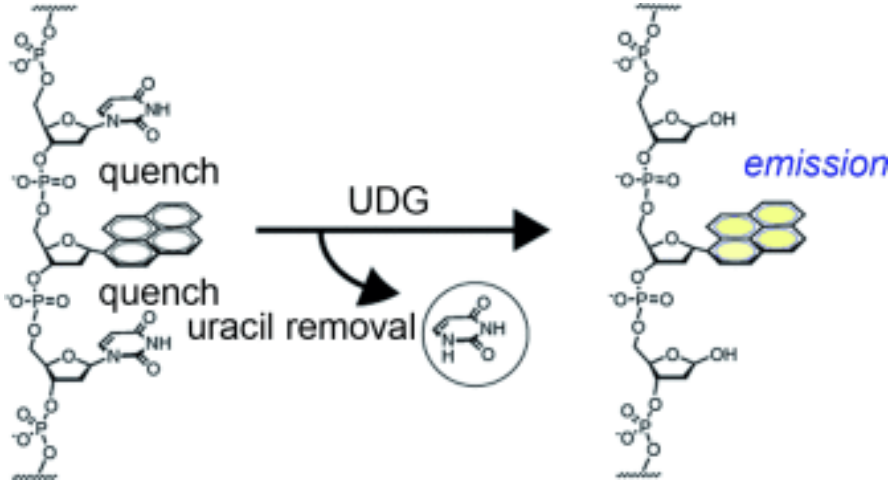


ACS Chem. Biol. 2018, 1721−1733.

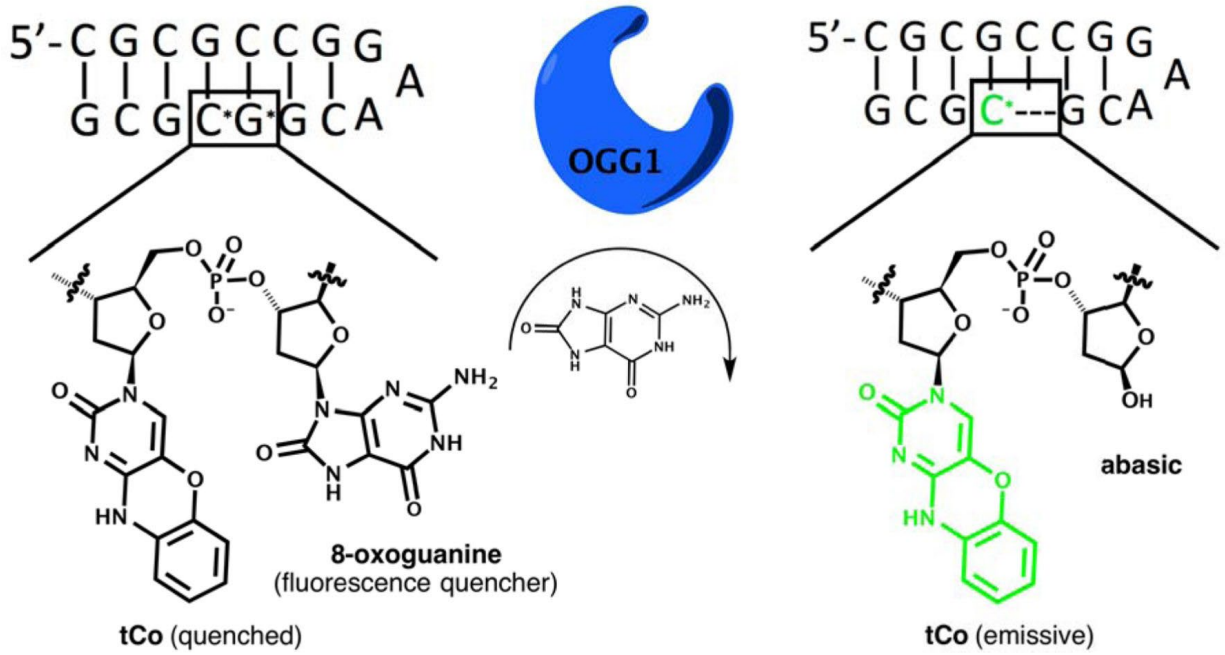


Major types of DNA repair probe designs. (a) Simple molecular beacon probe. (b) DNA binding-stimulated fluorescence probe. (c) Naturally quenched probe. (d) Host cell reactivation probe. (e) O6-guanine labeled probe. (f) Graphene oxide based probe.

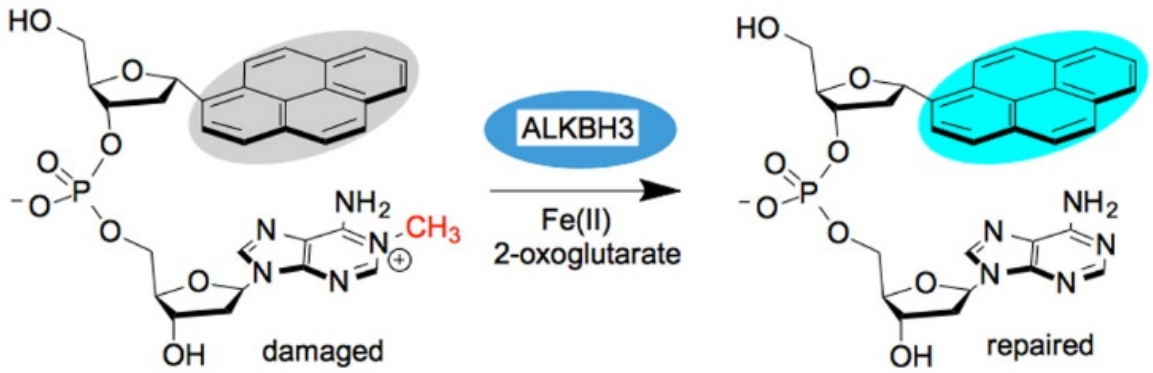
Turn-on fluorescent probes for DNA repair enzymes



Angew. Chem., Int. Ed. 2012, 1689−1692.



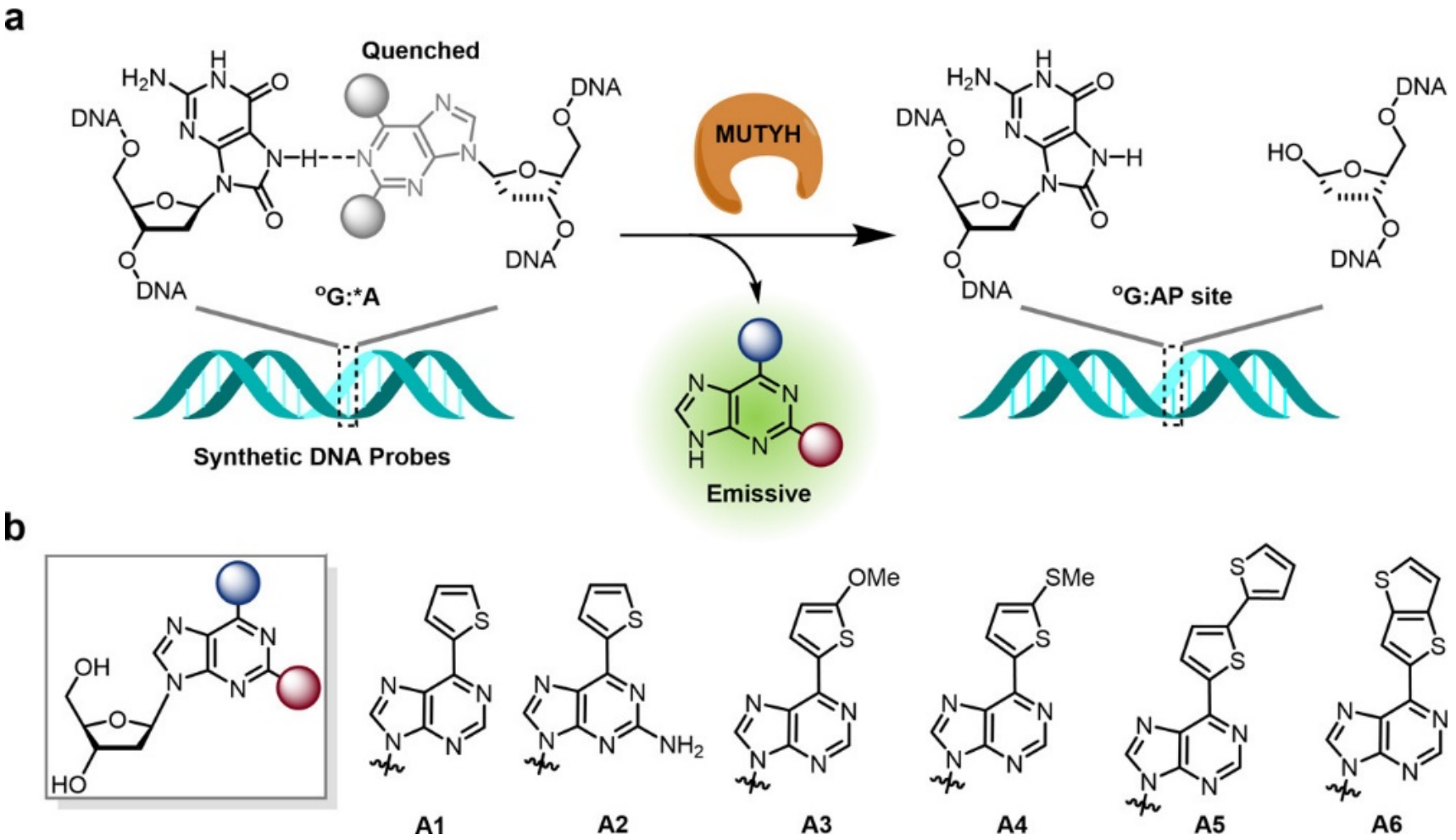
ChemBioChem 2015, 1637−1646.



J. Am. Chem. Soc. 2016, 3647−3650.

MUTYH酶切除的是未受损的腺嘌呤而不是受损的碱基，而且腺嘌呤对于大多数荧光团来说通常是一种较差的猝灭剂。

DNA-based light-up fluorescent probes



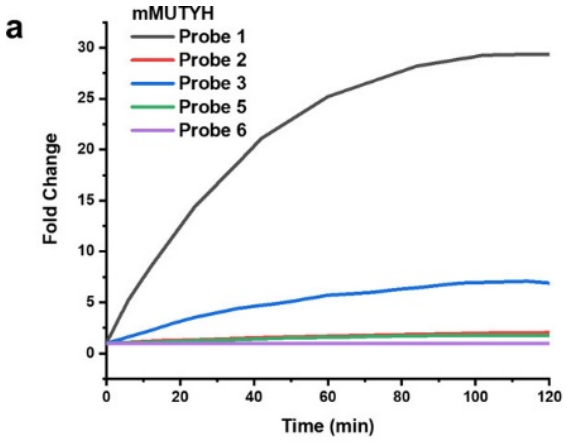
（i）具有相对长的激发/发射波长和足够亮度的有效荧光团；
（ii）被相邻的DNA碱基或°G有效猝灭；
（iii）被MUTYH有效识别和切除

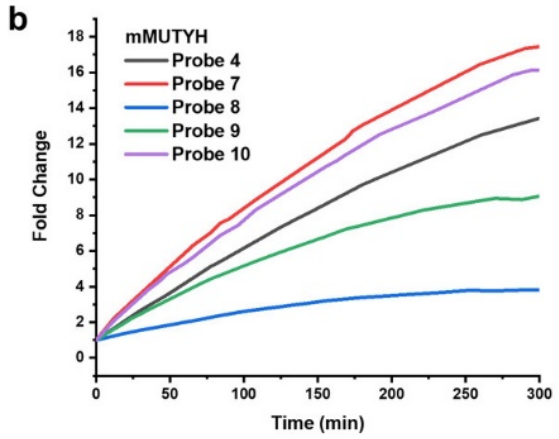
Figure 1. Design of DNA-based light-up fluorescent probes for adenine excision by MUTYH. (a) Neighboring DNA bases quench the emission of a fluorescent adenine analogue, which is excised by MutY/MUTYH, resulting in a turn-on signal. (b) Fluorescent adenine analogues designed and tested in this study.

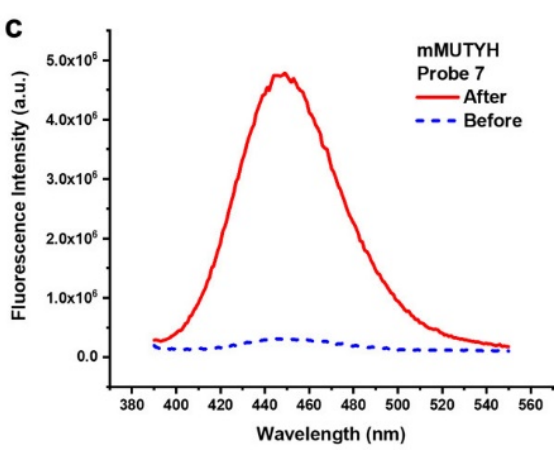
Probe Designs for Fluorogenic MUTYH Reporting

Probe	Probe Sequence[a]	$\lambda_{Ex/Em}$ (nm)	Fold Change[b]
1	5′-TCGAGCTA1TTAGCTCGAAGAGCTAA°GAGCTCGA-3′	324/380	29.4
2	5′-TCGAGCTA2TTAGCTCGAAGAGCTAA°GAGCTCGA-3′	348/434	2.1
3	5′-TCGAGCTA3TTAGCTCGAAGAGCTAA°GAGCTCGA-3′	360/416	6.9
4	5′-TCGAGCTA4TTAGCTCGAAGAGCTAA°GAGCTCGA-3′	372/454	13.4
5	5′-TCGAGCTA5TTAGCTCGAAGAGCTAA°GAGCTCGA-3′	380/473	1.8
6	5′-TCGAGCTA6TTAGCTCGAAGAGCTAA°GAGCTCGA-3′	358/422	1.0
7	5′-GAGCTA4TTAGCGAAGCTAA°GAGCTC-3′	372/454	17.4
8	5′-GCTA4TTAGAATAA°GAGC-3′	372/454	3.8
9	5′-GAGCAA4ATAGCGAAGCTAT°GTGCTC-3′	372/454	9.1
10	5′-$G_{OMe}A_{OMe}$GCTA4TTAGCGAAGCTAA°GAGC$U_{OMe}C_{OMe}$-3′	372/454	16.1
11[c]	5′-$G_{OMe}A_{OMe}$GCTATTAGCGAAGCTAA°GAGC$U_{OMe}C_{OMe}$-3′	-	-
12[d]	5′-$G_{OMe}A_{OMe}$GCTATTAGCGAAGCTAATAGC$U_{OMe}C_{OMe}$-3′	-	-

Photophysical properties of the fluorescent adenine derivatives







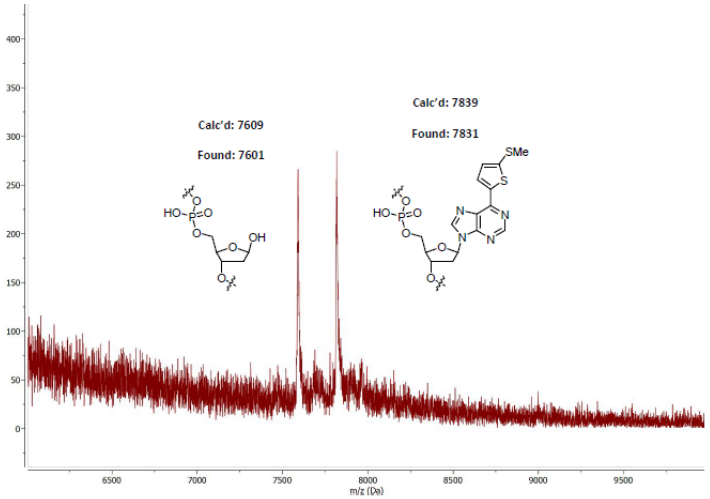
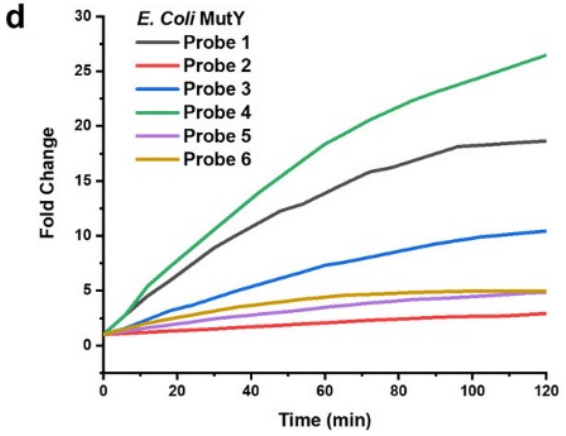
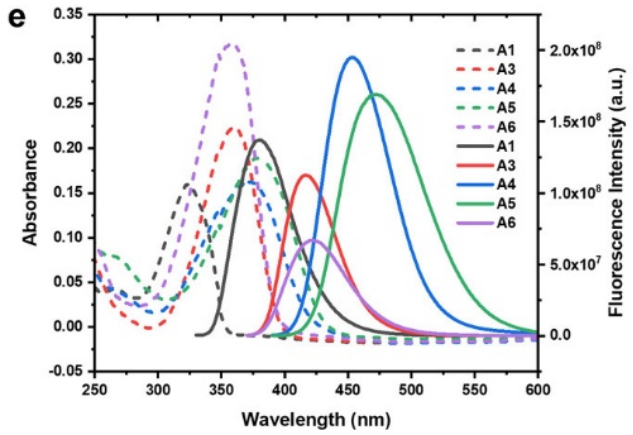
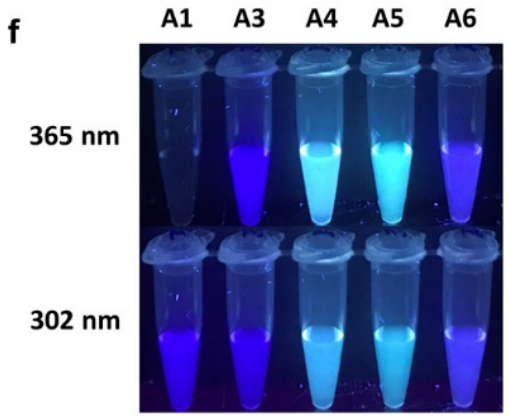


Figure S4. MALDI-TOF Analysis of reaction products of **FMART** probe **7**.



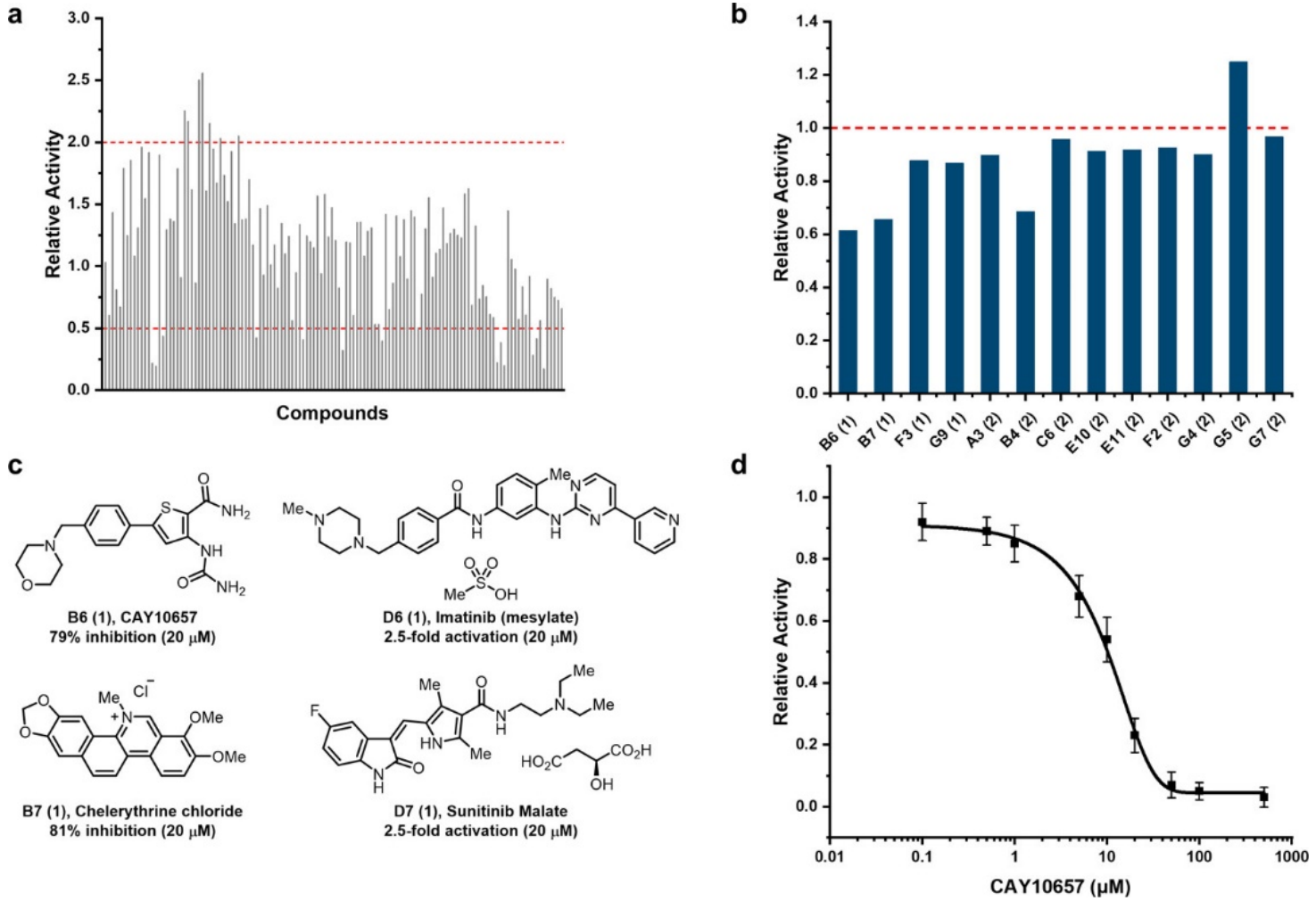




Nucleoside	$\lambda_{Ex/Em}$ (nm)	Stokes shift (nm)	Molar extinction coefficient ($M^{-1}cm^{-1}$)	Quantum yield of nucleosides	Quantum yield of probes
A1	324/380	56	21000	0.25	0.0024 (probe 1)
A2	348/434	86	11200	0.41[6]	0.0069 (probe 2)
A3	360/416	56	29800	0.13	0.0051 (probe 3)
A4	372/454	82	27400	0.37	0.0070 (probe 4)
A5	380/473	93	32000	0.35	0.035 (probe 5)
A6	358/422	64	31800	0.063	0.0038 (probe 6)

Figure 2. Kinetics of fluorescent FMART probe responses: (a) probes 1–3 and 5–6 or (b) probes 4 and 7–10 (440 nM) incubated with mMUTYH (220 nM), 20 mM Tris HCl pH 7.5, 10 mM ethylenediaminetetraacetic acid (EDTA), and 30 mM NaCl at 37 °C, measured at their emission maxima listed in Table S1. (c) Fluorescence emission spectra of probe 7 before and after 5 h of incubation with mMUTYH. (d) Kinetics of probes 1–6 (600 nM) incubated with Escherichia coli MutY (300 nM), 20 mM Tris HCl pH 7.5, 10 mM EDTA, and 30 mM NaCl at 37 °C, measured at their emission maxima. (e) Absorption and emission spectra of new nucleosides A1 and A3–5 (10 µM) in PBS (pH 7.4, room temperature). (f) Images of solutions of new nucleosides A1 and A3–5 (10 µM) in PBS (pH 7.4, room temperature) above a transilluminator at two excitation wavelengths.

Application of FMART probes to small-molecule screening



缺乏适合高通量筛选的分析

Figure 3. Application of FMART probes to small-molecule screening. (a) Mammalian repair enzyme activity data from screening a small-molecule library using probe 7 in a 384-well plate (excitation 355 nm, emission monitored at 460 nm). Compounds (20 μM) were incubated with mMUTYH (200 nM) at 37 °C for 10 min before probe 7 (400 nM) was added. (b) Validation of 13 inhibitor candidates at 5 μM. (c) Structures of representative small-molecule modulator candidates. (d) Dose–response curve of CAY10657 with mMUTYH, measured with probe 7.

The FMART probes in cellular contexts

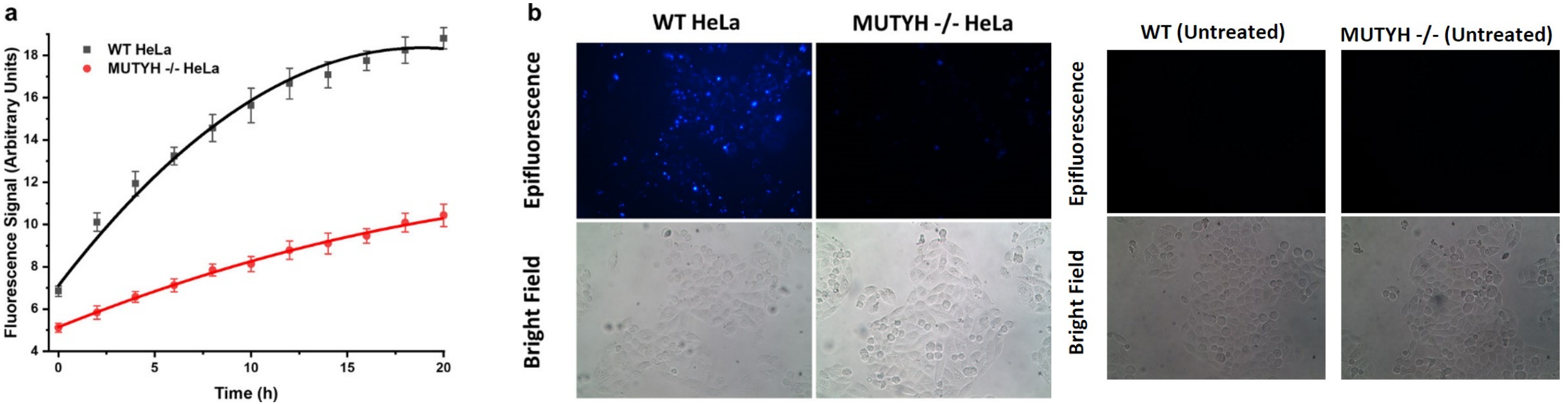


Figure 4. (a) Time course of FMART probe 10 response (500 nM) in HeLa cell lysates. (b) Imaging of HeLa cells with FMART probe 10. Note selective fluorescence signals in WT cells as compared with those lacking the MUTYH protein.

FMART探针可用于细胞提取物以及活细胞中MUTYH活性的测量和成像。

The FMART probes in more clinically relevant applications

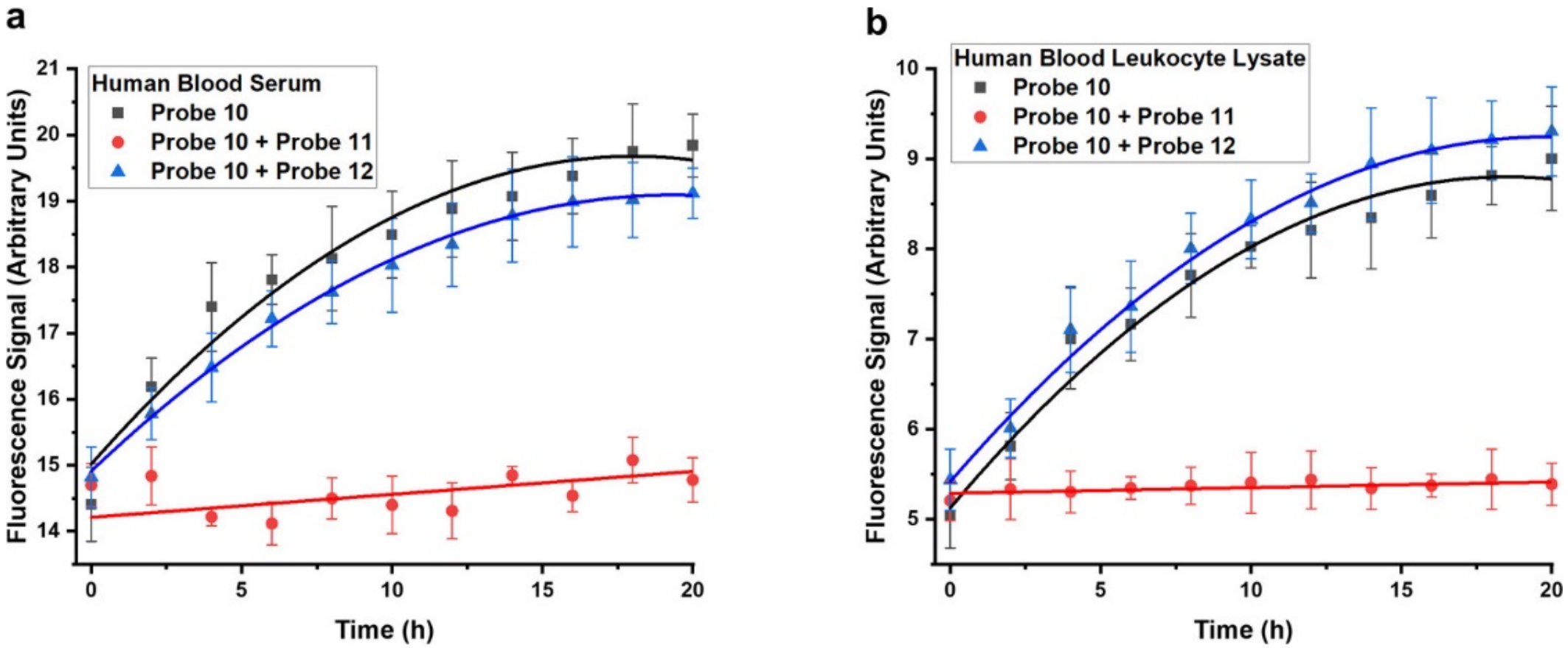


Figure 5. (a) Time course of FMART probe 10 response (500 nM) in human blood serum with or without competing substrate 11 (2 μM) or 12 (2 μM). (b) Time course of FMART probe 10 response (500 nM) in human blood leukocyte lysate with or without competing substrate 11 (2 μM) or 12 (2 μM). The fluorescence was monitored at 460 nm (355 nm excitation). Error bars show standard deviation from three replicates.