UC Davis
UC Davis Previously Published Works

Title

Designer Fluorescent Adenines Enable Real-Time Monitoring of MUTYH Activity

Permalink

https://escholarship.org/uc/item/97n422n3

Journal

ACS Central Science, 6(10)

ISSN

2374-7943

Authors

Zhu, Ru-Yi
Majumdar, Chandrima
Khuu, Cindy
et al.

Publication Date

2020-10-28

DOI

10.1021/acscentsci.0c00369

Peer reviewed

Designer Fluorescent Adenines Enable Real-Time Monitoring of MUTYH Activity

Ru-Yi Zhu, Chandrima Majumdar, Cindy Khuu, Mariarosaria De Rosa, Patricia L. Opresko, Sheila S. David, and Eric T. Kool*

ACCESS | Metrics & More | Article Recommendations | Supporting Information

ABSTRACT: The human DNA base excision repair enzyme MUTYH (MutY homolog DNA glycosylase) excises undamaged adenine that has been misincorporated opposite the oxidatively damaged 8-oxoG, preventing transversion mutations and serving as an important defense against the deleterious effects of this damage. Mutations in the *MUTYH* gene predispose patients to MUTYH-associated polyposis and colorectal cancer, and MUTYH expression has been documented as a biomarker for pancreatic cancer. Measuring MUTYH activity is therefore critical for evaluating and diagnosing disease states as well as for testing this enzyme as a potential therapeutic target. However, current methods for measuring MUTYH activity rely on indirect electrophoresis and radioactivity assays, which are difficult to implement in biological and clinical settings. Herein, we synthesize and identify novel fluorescent adenine derivatives that can act as direct substrates for excision by MUTYH as well as bacterial MutY. When incorporated into synthetic DNAs, the resulting fluorescently modified adenine-release turn-on (FMART) probes report on enzymatic base excision activity in real time, both *in vitro* and in mammalian cells and human blood. We also employ the probes to identify several promising small-molecule modulators of MUTYH by employing FMART probes for *in vitro* screening.
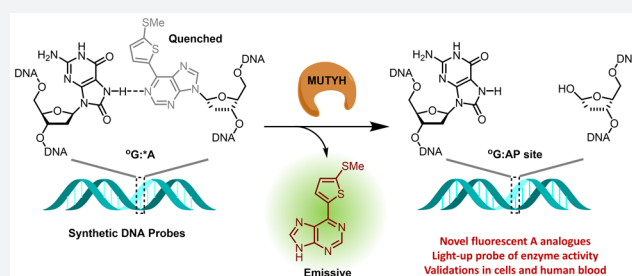
Oxidative DNA damage arising from the attack of both endogenous and exogenous reactive oxygen species (ROS) is a major contributor to a range of diseases, including cancer.[1] Among the varied oxidatively induced lesions that occur in DNA, 8-oxo-7,8-dihydroguanine (8-oxoG or °G) is the most prevalent, and frequently causes G:C to T:A transversion mutations.[2] Three human enzymes, OGG1, MTH1, and MUTYH (MutY homolog DNA glycosylase), are responsible for repairing 8-oxoG-related lesions. OGG1 excises 8-oxoG from an °G:C base pair in DNA, while MTH1 hydrolyzes °GTP to °GMP, thus preventing its incorporation into DNA during replication. However, when 8-oxoG escapes surveillance by MTH1 and OGG1, its miscoding nature leads it to be preferentially paired with an adenine during DNA replication, forming a °G:A base mispair. This hazardous mismatch is recognized, and the undamaged adenine is excised by MUTYH as the first chemical step in base excision repair (BER). MUTYH is a monofunctional glycosylase that remains tightly bound to the abasic site created by adenine excision and relies on the endonuclease APE1 for removal of the abasic sugar. Downstream BER enzymes restore the correct cytosine-pairing partner and excise 8-oxoG. Multiple cellular isoforms of MUTYH exist, directing repair both in chromosomal and mitochondrial DNA.[3]

The repair activity of MUTYH is particularly important in preventing G:C to T:A transversion mutations in human populations. Approximately 1 to 2% of the population carries a mutation in one copy of the *MUTYH* gene, which is linked to various human diseases. Individuals with inherited biallelic mutations in the *MUTYH* gene, encoding a poorly functional MUTYH glycosylase, are correlated with a colorectal cancer predisposition syndrome known as MUTYH-associated polyposis (MAP). Of note, individuals with a single-allele mutation have intermediate risks of MAP.[4,5] The sequencing of the adenomatous polyposis coli (*APC*) gene from the tumors of these patients reveals a high proportion of G:C to T:A transversion mutations, suggesting that the lack of fully functional MUTYH causes the accumulation of mutations. Thus, it is possible that upregulating MUTYH activity may be a useful therapeutic strategy for preventing the onset and progression of cancer in individuals harboring MUTYH variants.[6] Interestingly, Phillips et al. showed that the suppression of wild-type (WT) MUTYH by siRNA in a panel of pancreatic cancer cell lines reduced pancreatic cancer cell growth and increased chemosensitivity.[7] In addition to the enzyme's relevance to cancer, mice lacking the *MUTYH* gene
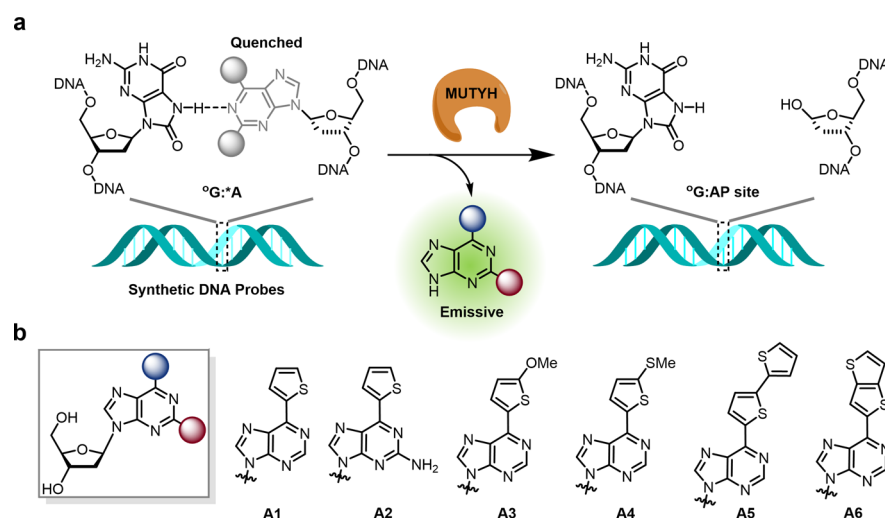
Figure 1. Design of DNA-based light-up fluorescent probes for adenine excision by MUTYH. (a) Neighboring DNA bases quench the emission of a fluorescent adenine analogue, which is excised by MutY/MUTYH, resulting in a turn-on signal. (b) Fluorescent adenine analogues designed and tested in this study.

Table 1. Probe Designs for Fluorogenic MUTYH Reporting

Probe	Probe Sequence[a]	$\lambda_{Ex/Em}$ (nm)	Fold Change[b]
1	5'-TCGAGCT**A1**TTAGCTC**GAA**GAGCTAA**°G**AGCTCGA-3'	324/380	29.4
2	5'-TCGAGCT**A2**TTAGCTC**GAA**GAGCTAA**°G**AGCTCGA-3'	348/434	2.1
3	5'-TCGAGCT**A3**TTAGCTC**GAA**GAGCTAA**°G**AGCTCGA-3'	360/416	6.9
4	5'-TCGAGCT**A4**TTAGCTC**GAA**GAGCTAA**°G**AGCTCGA-3'	372/454	13.4
5	5'-TCGAGCT**A5**TTAGCTC**GAA**GAGCTAA**°G**AGCTCGA-3'	380/473	1.8
6	5'-TCGAGCT**A6**TTAGCTC**GAA**GAGCTAA**°G**AGCTCGA-3'	358/422	1.0
7	5'-GAGCT**A4**TTAGC**GAA**GCTAA**°G**AGCTC-3'	372/454	17.4
8	5'-GCT**A4**TTA**GAA**TAA**°G**AGC-3'	372/454	3.8
9	5'-GAGC**A**A4ATAGC**GAA**GCTAT**°G**TGCTC-3'	372/454	9.1
10	5'-G_{OMe}A_{OMe}GCT**A4**TTAGC**GAA**GCTAA**°G**AGCU_{OMe}C_{OMe}-3'	372/454	16.1
11[c]	5'-G_{OMe}A_{OMe}GCT**A**TTAGC**GAA**GCTAA**°G**AGCU_{OMe}C_{OMe}-3'	-	-
12[d]	5'-G_{OMe}A_{OMe}GCT**A**TTAGC**GAA**GCTAA**T**AGCU_{OMe}C_{OMe}-3'	-	-

[a]Nucleobases highlighted in red are *A:8-oxoG base pairs. The GAA loop is highlighted in blue. Nucleobases highlighted in green show varied neighboring bases. [b]Fold change is calculated as FI (after repair)/FI (before repair), FI denotes fluorescence intensity. [c]Probe **11** is a native substrate for MUTYH used as a competitor in cellular studies. [d]Probe **12** is an undamaged substrate used as a competitor in cellular studies.

have reduced inflammatory responses, suggesting that inhibitors may be useful in clinically addressing inflammation.[8] Moreover, measuring MUTYH may also serve as a clinically useful biomarker: Sugimura et al. found that MUTYH is highly downregulated in most human gastric cancer cell lines and its low expression level was a predictor of a poor prognosis in gastric cancer.[9] These clinical and preclinical findings highlight the importance of measuring the MUTYH repair activity.

Despite the important biological and potential therapeutic implications of MUTYH, methods to monitor the activity levels of this enzyme in real time are lacking. Currently, the major methods for measuring MUTYH activity are electrophoresis and radiation-based assays,[10] which are indirect and laborious and are mostly done *in vitro* with limited utility for therapeutic or biomedical settings. The lack of a simple assay for base excision by MUTYH prompted us to develop fluorogenic probes that may be amenable for quantitatively

monitoring its real-time activity levels *in vitro* as well as in cells and cell lysates. In addition, simple light-up fluorescent probes would enable high-throughput screening (HTS) to identify inhibitors and/or activators of the enzyme, which can serve as tools for testing therapeutic hypotheses concerning the enzyme.

Our new probe design takes a distinct approach from prior fluorescence reporters of DNA repair.[11] Previous work has described turn-on fluorescent probes for DNA repair enzymes in which the damaged bases (e.g., uracil, 8-oxoG, and *N*1-methyladenosine) act as fluorescence quenchers for neighboring fluorophores.[12–15] In such designs, fluorophores in the DNA remain dark before repair and become emissive after removal of the nearby damage by DNA repair enzymes. However, this strategy is not readily applicable to MUTYH, since the enzyme excises the undamaged adenine rather than a damaged base, and adenine is typically a poor quencher for
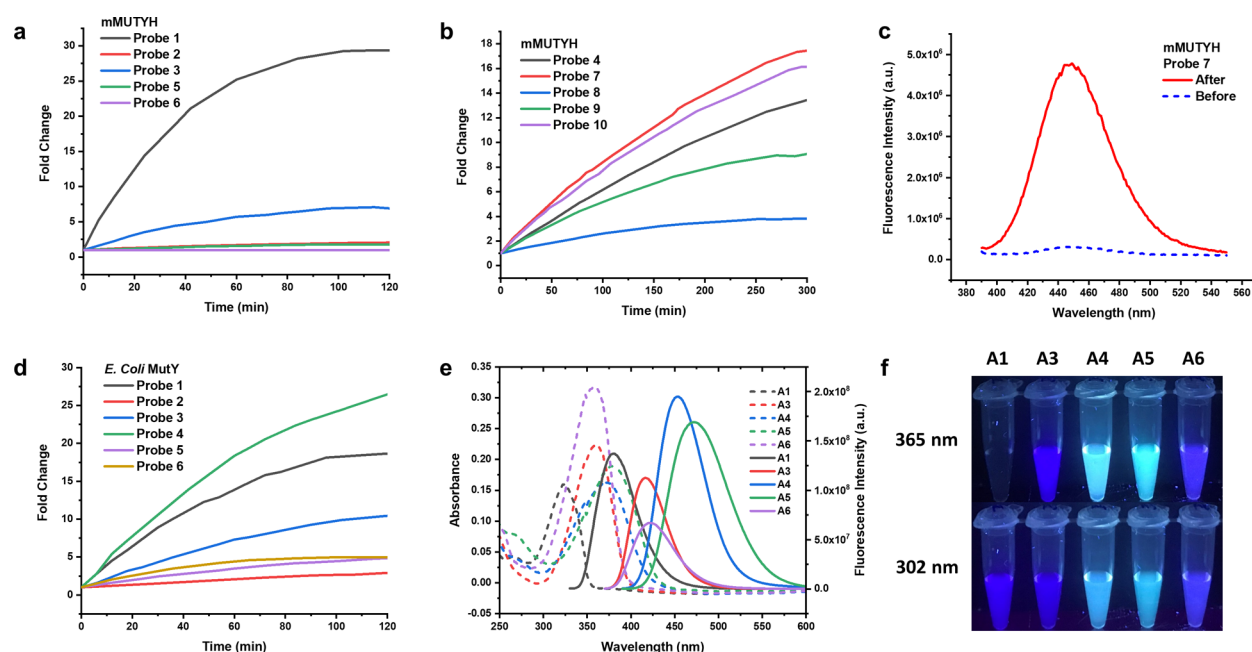
Figure 2. Kinetics of fluorescent FMART probe responses: (a) probes **1−3** and **5−6** or (b) probes **4** and **7−10** (440 nM) incubated with mMUTYH (220 nM), 20 mM Tris HCl pH 7.5, 10 mM ethylenediaminetetraacetic acid (EDTA), and 30 mM NaCl at 37 °C, measured at their emission maxima listed in Table 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.

most fluorophores.[16] Hence, we adopted a different strategy, by chemically synthesizing fluorescent purine derivatives that could mimic undamaged adenine in ᵒG:A mispairs. In principle, such a derivative might be quenched by carefully chosen neighboring DNA bases and, if it successfully served as an enzymatic excision substrate, would then become brightly emissive upon its release into solution (Figure 1a).

Here, we report the development of fluorescently modified adenine-release turn-on (FMART) probes for MUTYH and bacterial MutY, which were developed by addressing three major challenges. For utility, the analogue needs to be (i) an effective fluorophore having relatively long excitation/emission wavelengths and sufficient brightness; (ii) effectively quenched by neighboring DNA bases and/or ᵒG; and (iii) recognized and excised by MUTYH efficiently. As for the last point, although there exists little information regarding MUTYH's unnatural substrate specificity, we were able to draw from a crystal structure of bacterial MutY bound to the substrate as well as its substrate specificity for modified adenines.[17,18] The data indicates that modifications of the 6-amino group are tolerated by the bacterial enzyme, which shares significant homology to the mammalian enzyme. Therefore, we replaced the 6-amino group of adenine with aromatic rings to confer longer-wavelength fluorescence.[19] We chose thiophene and its derivatives because of their relatively small sizes and useful photophysical properties. Thiophene rings (Figure 1b) were introduced through a Pd-catalyzed Stille coupling between protected 6-chloroadenosine and thienyl tin reagents (see SI for details). We synthesized a series of 6-thienyl-adenosine phosphoramidite derivatives and incorporated them into DNA probes that were designed to form hairpin structures (Table 1). We initially tested the approach with 6-thienyl-purine (**A1**) incorporated into a 33mer hairpin DNA, designed to form a 15

bp duplex and harboring a central **A1**:ᵒG base pair with **A1** sandwiched by two thymines as potential quenching residues in the adjacent pairs. Due to challenges associated with obtaining a high amount of active human MUTYH (hMUTYH), we chose the mouse homologue (mMUTYH), which expresses with higher levels of activity and shares >90% sequence identity with human MUTYH for *in vitro* validation of our probes.[20] Incubation of FMART probe **1** (440 nM) and mMUTYH (220 nM) at 37 °C resulted in an immediate fluorescence increase and a 29-fold fluorescence enhancement at 2 h, indicating that the 6-thienyl substitution is well-tolerated by mMUTYH (Figure 2a) and that the fluorescent base is well-quenched in this sequence context. However, FMART probe **1** is expected to have limited utility in cells due to the short excitation/emission wavelength of **A1**. 2-Amino-6-thienyl purine deoxyribonucleoside (**A2**) has been reported to emit at 434 nm with moderate brightness.[21] **A2** was incorporated into the same 33mer hairpin DNA and tested against mMUTYH; in this case, we observed a very slow fluorescence response over 2 h with a modest 2.1-fold light-up signal, suggesting that the 2-amino group significantly suppresses the enzyme activity; this is consistent with the documented ability of MUTYH to discriminate between guanine (which has a 2-amino group) and adenine.[22] Thus, we focused on increasing emission wavelength instead by modifying the thiophene ring with electron-donating groups. We first synthesized 6-(2-methoxy-thienyl)-purine deoxyribonucleoside (**A3**) and found that the methoxy group indeed red-shifts the emission in **A3** to 420 nm. FMART probe **3** was assembled and tested in the presence of mMUTYH, yielding a 6.9-fold fluorescence increase after 2 h (Figure 2a). We attribute the lower fluorescence increase of probe **3** (as compared with **1**) to two possible factors: (i) **A1** is better
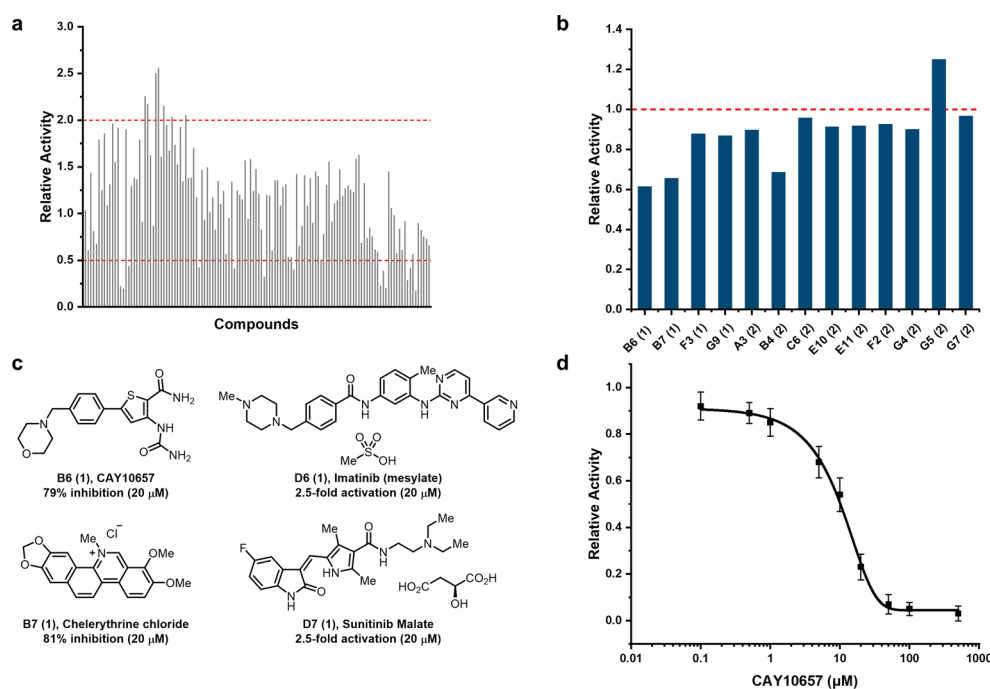
Figure 3. Application of FMART probes to small-molecule screening. (a) Mammalian repair enzyme activity data from screening a small-molecule library using probe **7** in a 384-well plate (excitation 355 nm, emission monitored at 460 nm). Compounds (20 μM) were incubated with mMUTYH (200 nM) at 37 °C for 10 min before probe **7** (400 nM) was added. See details in Supporting Information. (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**.

quenched by neighboring bases than **A3** (Table S2), and (ii) the larger size of **A3** slightly disfavors MUTYH recognition and activity. To address the low photophysical performance of probe **3**, we replaced the methoxy group with a methylthio group, which is known to enhance photophysical properties without large steric alterations.[23] Indeed, the maximal emission wavelength of **A4** was further red-shifted to 454 nm, and a relatively robust 13.4-fold turn-on signal was obtained after 5 h (Figure 2b). Further increases in size (in probes containing fluorescent analogues **A5** and **A6**) were demonstrated to be deleterious, as probes **5** and **6** failed to act as substrates of mMUTYH (Figure 2a). In contrast, *Escherichia coli* MutY proved to be less sensitive to the size of 6-substituents of adenine (Figure 2d), giving comparable or better turn-on signals for all probes in a shorter time; however, the largest fluorescent adenine derivatives remained relatively less efficient substrates than analogue **A4**. Overall, 6-(2-methylthiothiophenyl)purine analogue **A4** proved to have the most useful combination of long-wavelength fluorescence and enzymatic substrate ability.

To explore the DNA substrate length preference of MUTYH, we compared three FMART probes containing analogue **A4** (i.e., **4**, **7**, and **8**) in hairpin DNAs with different duplex lengths. The 11 bp hairpin DNA probe **7** responded with a slightly higher reaction rate than 15 bp probe **4**, whereas the smallest 7 bp probe **8** reacted much more slowly (Figure 2b). Lastly, we tested the effect of bases neighboring **A4** by switching adjacent thymines with adenines (probe **9**); this reduced the turn-on signal to 9.1-fold, presumably due to less quenching effectiveness of adenine. To confirm that the fluorescence light-up was due to the enzymatic release of the modified adenine, we successfully detected both unreacted probe **7** and its abasic form by matrix-assisted laser desorption/

ionization−time of flight (MALDI-TOF) mass spectrometry (Figure S3), which unambiguously confirmed that **A4** was excised and released by MUTYH. It should be noted that both MUTYH and bacterial MutY do not turn over *in vitro* or *in vivo* but rather remain tightly bound to the product DNA;[24,25] thus, all probes are not fully converted to products under these conditions. As a result, our light-up signal measurements are likely underestimates of the complete probe response.

Finally, we characterized photophysical properties of the new fluorescent adenine derivatives prepared for this study (Figure 2e, 2f, and Table S2), of which the deoxynucleosides of **A3−6** were previously unknown. In general, installation of electron-donating groups on the thiophene ring red-shifts excitation/emission wavelengths and increases quantum yields (except **A3** and **A6**), with **A4** and **A5** as the brightest fluorescent adenine analogues, having quantum yields of 0.37 and 0.35, respectively. All adenine derivatives were strongly quenched by neighboring bases except **A5**, which was quenched by a smaller degree (Table S2). Given the broad utility of fluorescent DNA base analogues, these compounds may prove to be useful in many other nucleic acid-related applications.

Since regulation of MUTYH activity has been shown to be associated with multiple biological pathways and serious disease states,[7−9] the enzyme has been considered a potential therapeutic target. However, to the best of our knowledge, no small molecule modulator of MUTYH activity is yet known, possibly due to the lack of an assay amenable to high-throughput screening. In an initial test of whether the new FMART probe design could be applicable to library screening, we employed probe **7** in an assay with mMUTYH with a 160-member library of small-molecule biologically active compounds in 384-well plate mode in a luminescence plate reader.
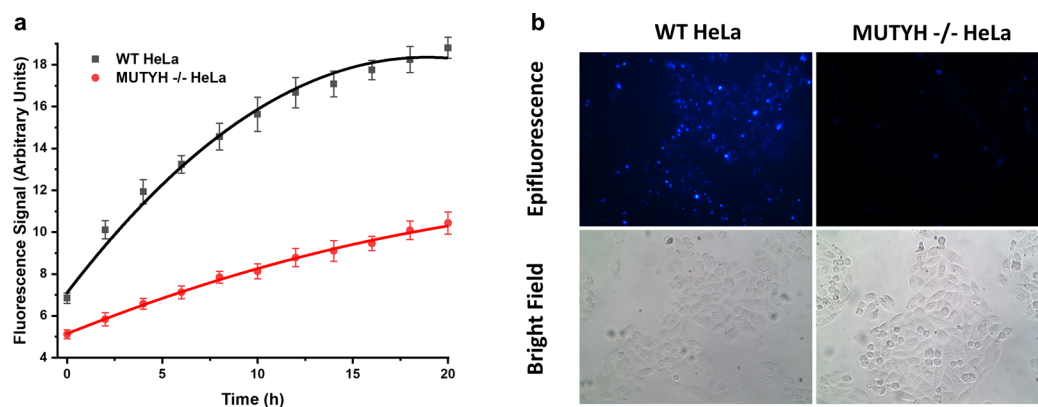
Figure 4. (a) Time course of FMART probe **10** response (500 nM) in HeLa cell lysates. (b) Imaging of HeLa cells with FMART probe **10**. Note selective fluorescence signals in WT cells as compared with those lacking the MUTYH protein. See Figure S5 for larger images.
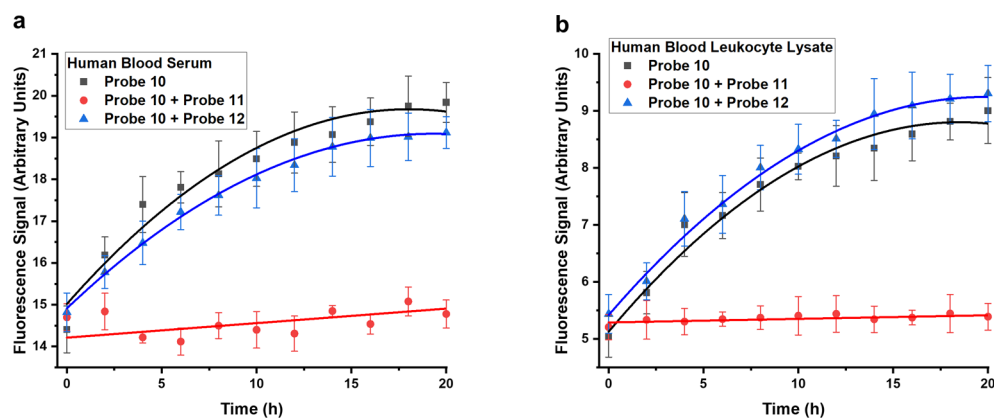

Figure 5. (a) Time course of FMART probe **10** response (500 nM) in human blood serum with or without competing substrate **11** (2 μM) or **12** (2 μM). (b) Time course of FMART probe **10** response (500 nM) in human blood leukocyte lysate with or without competing substrate **11** (2 μM) or **12**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

We incubated the compounds (20 μM) with mMUTYH (200 nM) at 37 °C for 10 min before probe **7** (400 nM) was added, and then, the fluorescence was monitored at 460 nm immediately after the addition of the probe. The relative enzyme activity was calculated by comparing the initial rate in the presence of a compound with the DMSO-only control (Figure 3a). Among the 160 compounds surveyed at 20 μM, we were able to identify several promising inhibitor and activator candidates for mMUTYH. We further validated the inhibitory effect of selected inhibitors at 5 μM by comparing the initial rates for the first 30 min (Figure 3b), and identified a small number of compounds with moderate apparent inhibition and activation activities (Figure 3c). Selecting among the inhibitors, we were able to use the FMART probe assay to quantify the relative inhibitory activity of CAY10657 at varied concentrations, yielding an apparent IC_{50} value of 10.2 μM (Figure 3d). Thus, we conclude that FMART probes are readily applicable to *in vitro* assays in screening for modulators of MUTYH activity.

In order to test the utility of FMART probes in cellular contexts, we synthesized the nuclease-protected FMART probe **10** by substituting the first two nucleosides of both 5′- and 3′-ends with 2′-OMe groups. FMART probe **10** showed a very similar response as unprotected probe **7** with purified mMUTYH *in vitro* (Figure 2b). Although mMUTYH has high sequence homology to hMUTYH, it is necessary to test whether FMART probes can indeed be used to measure

hMUTYH activity, which could be especially useful in studying the biology of DNA repair in human cell lines.

Nuclear and mitochondrial forms of hMUTYH are ubiquitously expressed in cell lines and localize in nuclei and cytoplasm, respectively, and studies have shown that the majority of MUTYH exists in the cytoplasm of HeLa cells.[26] First, we set out to examine the performance of probe **10** in the cytosolic extract of WT HeLa cells. Incubation of 500 nM probe **10** in WT HeLa cytosolic lysate at 37 °C resulted in an observable fluorescence increase over an extended period of time (Figure 4a), giving a reproducible 3-fold turn-on fluorescence signal. To confirm that the generated signal arises mainly from the activity of hMUTYH, we also tested FMART probe **10** in MUTYH (−/−) HeLa cytosolic extract under identical conditions. Only a small increase in the fluorescence (ca. 7.2-fold lower in initial rate) was observed over the time course in this control experiment, confirming that the signal in WT cells is mainly due to MUTYH activity and validating the expected selectivity of the probe. We attribute the small background fluorescence increase in MUTYH (−/−) HeLa cytosolic extract to the possible slow digestion of probe **10** by nuclease enzymes. We also tested the ability of the nuclease-protected probe to respond in living HeLa cells, transfecting it with lipofectamine 2000 and imaging by epifluorescence microscopy (Figure 4b). With WT HeLa cells, the probe yields diffuse blue/cyan fluorescence in the cells (Figure 4b). Closer examination shows the signals localized primarily in the

cytoplasm of the cells (Figure S5), consistent with the literature observation of highest MUTYH expression in the cytosol,[26] but also explainable by limited delivery of the probe into the nucleus by the transfection reagent. Importantly, images of control HeLa cells lacking MUTYH protein show only very weak fluorescence, similar to cells in which no probe was added (Figure S6). Thus, we conclude that FMART probes can be employed in cellular extracts as well as in living cells to measure and image relative MUTYH activity. The new probe design offers a convenient and rapid alternative to a prior plasmid-based assay of intracellular MUTYH activity.[27] While previous clinical studies cited expression levels of MUTYH as measured by mRNA levels and Western blots in patient samples, varied expression levels do not always correlate well with actual enzymatic activity, due to variations in translation levels, to different splice variants, and to variable post-translational modifications. Thus, the new probe design offers a previously unavailable approach for evaluating actual enzymatic activity, which is expected to be more relevant to disease states.

To further test the potential utility of FMART probes in more clinically relevant applications, we evaluated the nuclease-protected probe **10** in preparation of human whole blood. Although quantification of MUTYH in human blood serum has been done via enzyme-linked immunosorbent assays,[28] it is critical to determine MUTYH activity rather than quantity for disease relevance (e.g., MAP). To the best of our knowledge, the evaluation of MUTYH activity in human blood as a potential diagnostic tool has not been realized. Serum was prepared from fresh human whole blood and then diluted 7-fold with reaction buffer (see Supporting Information for details), followed by the addition of FMART probe **10** (500 nM) and incubation at 37 °C. Fluorescence increases over a period of 20 h were observed (Figure 5a). To confirm that the fluorescence signal was due to MUTYH activity, we prepared an authentic substrate of MUTYH (probe **11**)-containing adenine as a competitive binding substrate. As MUTYH does not turn over, the addition of excess probe **11** should outcompete probe **10** in reacting with MUTYH, resulting in lower fluorescence. Indeed, the presence of this competitor resulted in only a very small fluorescence increase, and differences between the two were clear after only 3−4 h (Figure 5a), suggesting high selectivity. However, it could be argued that other non-oxoG-targeting enzymes (such as nucleases) might also be inhibited with competitor probe **11**. To further test this selectivity, we prepared competitor probe **12** by replacing 8-oxoG in probe **11** with thymine. The addition of excess probe **12** had almost no effect on signals from probe **10** (Figure 5a), indicating clearly that the inhibition effect of competitor probe **11** requires the presence of its 8-oxoG/A pair, excluding the possibility that the fluorescence increase was due to nucleases (Figure S8). To further test the selectivity of fluorescent probe **10**, we tested its responses with several other DNA repair glycosylases *in vitro*. No noticeable fluorescence change was observed for the other glycosylases (see Figure S8), indicating that probe **10** is indeed selective toward MUTYH among other DNA glycosylases. Overall, the data indicate that probe **10** is able to selectively report on the MUTYH activity both *in vitro* and in human serum. We also isolated human leukocytes from heparin-treated blood by lysing the red blood cells (see Supporting Information for details). Similarly to the serum measurements, clear MUTYH-specific signals were seen. Thus, we conclude

that the FMART probes can potentially be useful in the analysis of this biomedically important enzyme in authentic clinical specimens.

In summary, we have described a novel fluorescent probe architecture that enables the real-time measurement of the activity of a DNA repair enzyme that excises undamaged bases in the context of a DNA lesion. The FMART design makes use of novel fluorescent adenine analogues that act as direct enzyme substrates, being excised and released into solution by the enzyme. We have shown that the probe design functions well with mammalian forms of the enzyme as well as the bacterial homologue. We have successfully employed a FMART probe in a convenient *in vitro* screening assay format and further used the probe to discover and characterize the apparent inhibitory activity of a lead candidate small-molecule inhibitor. Finally, we have shown that a nuclease-protected form of the probe can effectively and selectively report on MUTYH activity in human cell lysates and that the relative activity can be imaged in living cells as well as blood preparations. We expect that this simple probe design could be employed in many MutY/MUTYH-relevant applications. In addition, the novel fluorescent adenine analogues described herein may also be useful in future studies of nucleotides and nucleic acids.

■ ASSOCIATED CONTENT

ⓈⒾ Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acscentsci.0c00369.

> Probe sequences, MS data, and mMUTYH fluorescence responses; MALDI-TOF analysis of FMART probe **7**; photophysical properties of nucleosides **A1−6** and probes **1−6**; fluorescence emission spectra of probes studied *in vitro* for mMUTYH and *E. coli* MutY; MALDI-TOF analysis of reaction mixtures of FMART probe **7**; a presentative work-flow of probe synthesis; larger version of Figure 4b, main text, showing signals associated with MUTYH-positive HeLa cells after lipofectamine transfection of probe **7**; negative controls showing cell images of WT and MUTYH −/− HeLa cells without a probe; and probe specificity evaluation *in vitro* and in human blood samples (PDF)

■ AUTHOR INFORMATION

Corresponding Author

Eric T. Kool − *Department of Chemistry, ChEM-H Institute, and Stanford Cancer Institute, Stanford University, Stanford, California 94305, United States;* ⓞ orcid.org/0000-0002-7310-2935; Email: kool@stanford.edu

Authors

Ru-Yi Zhu − *Department of Chemistry, ChEM-H Institute, and Stanford Cancer Institute, Stanford University, Stanford, California 94305, United States;* ⓞ orcid.org/0000-0001-5908-2555

Chandrima Majumdar − *Department of Chemistry, and Biochemistry, Molecular, Cellular and Developmental Biology Graduate Group, University of California at Davis, Davis, California 95616, United States*

Cindy Khuu − *Department of Chemistry, and Biochemistry, Molecular, Cellular and Developmental Biology Graduate*

Group, University of California at Davis, Davis, California 95616, United States; ⊙ orcid.org/0000-0002-9144-0137

Mariarosaria De Rosa − *Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania 15261, United States; Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15261, United States*

Patricia L. Opresko − *Department of Environmental and Occupational Health, University of Pittsburgh Graduate School of Public Health, Pittsburgh, Pennsylvania 15261, United States; Hillman Cancer Center, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania 15261, United States*

Sheila S. David − *Department of Chemistry, and Biochemistry, Molecular, Cellular and Developmental Biology Graduate Group, University of California at Davis, Davis, California 95616, United States;* ⊙ orcid.org/0000-0001-5873-7935

Complete contact information is available at:
https://pubs.acs.org/10.1021/acscentsci.0c00369

Notes
The authors declare no competing financial interest.

■ REFERENCES

(1) Cooke, M. S.; Evans, M. D.; Dizdaroglu, M.; Lunec, J. Oxidative DNA damage: mechanisms, mutation, and disease. *FASEB J.* **2003**, *17*, 1195−1214.

(2) Boiteux, S.; Coste, F.; Castaing, B. Repair of 8-oxo-7,8-dihydroguanine in prokaryotic and eukaryotic cells: Properties and biological roles of the Fpg and OGG1 DNA N-glycosylases. *Free Radical Biol. Med.* **2017**, *107*, 179−201.

(3) Banda, D. M.; Nuñez, N. N.; Burnside, M. A.; Bradshaw, K. M.; David, S. S. Repair of 8-oxoG:A mismatches by the MUTYH glycosylase: mechanism, metals and medicine. *Free Radical Biol. Med.* **2017**, *107*, 202−215.

(4) Al-Tassan, N.; Chmiel, N. H.; Maynard, J.; Fleming, N.; Livingston, A. L.; Williams, G. T.; Hodges, A. K.; Davies, D. R.; David, S. S.; Sampson, J. R.; Cheadle, J. P. Inherited variants of MYH associated with somatic G:C–>T:A mutations in colorectal tumors. *Nat. Genet.* **2002**, *30* (2), 227−232.

(5) Raetz, A. G.; David, S. S. When you're strange: unusual features of the MUTYH glycosylase and implications in cancer. *DNA Repair* **2019**, *80*, 16−25.

(6) Alli, E.; Solow-Cordero, D.; Casey, S. C.; Ford, J. M. Therapeutic targeting of *BRCA1*-mutated breast cancers with agents that activate DNA repair. *Cancer Res.* **2014**, *74* (21), 6205−6215.

(7) Sharbeen, G.; Youkhana, J.; Mawson, A.; McCarroll, J.; Nunez, A.; Biankin, A.; Johns, A.; Goldstein, D.; Phillips, P. MutY-Homolog (MYH) inhibition reduces pancreatic cancer growth and increases chemosensitivity. *Oncotarget.* **2017**, *8* (6), 9216−9229.

(8) Casorelli, I.; Pannellini, T.; De Luca, G.; Degan, P.; Chiera, F.; Iavarone, I.; Giuliani, A.; Butera, A.; Boirivant, M.; Musiani, P.; Bignami, M. The Mutyh base excision repair gene influences the inflammatory response in a mouse model of ulcerative colitis. *PLoS One* **2010**, *5* (8), e12070.

(9) Shinmura, K.; Goto, M.; Suzuki, M.; Tao, H.; Yamada, H.; Igarashi, H.; Matsuura, S.; Maeda, M.; Konno, H.; Matsuda, T.; Sugimura, H. 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. *J. Pathol.* **2011**, *225* (3), 414−423.

(10) Nuñez, N. N.; Majumdar, C.; Lay, K. T.; David, S. S. Fe-S Clusters and MutY base excision repair glycosylases: purification, kinetics, and DNA affinity measurements. *Methods Enzymol.* **2018**, *599*, 21−68.

(11) Wilson, D. L.; Kool, E. T. Fluorescent probes of DNA repair. *ACS Chem. Biol.* **2018**, *13* (7), 1721−1733.

(12) Stivers, J. T.; Pankiewicz, K. W.; Watanabe, K. A. Kinetic mechanism of damage site recognition and uracil flipping by Escherichia coli uracil DNA glycosylase. *Biochemistry* **1999**, *38* (3), 952−963.

(13) Ono, T.; Wang, S.; Koo, C. K.; Engstrom, L.; David, S. S.; Kool, E. T. Direct fluorescence monitoring of DNA base excision repair. *Angew. Chem., Int. Ed.* **2012**, *51* (7), 1689−1692.

(14) Edwards, S. K.; Ono, T.; Wang, S.; Jiang, W.; Franzini, R. M.; Jung, J. W.; Chan, K. M.; Kool, E. T. In vitro fluorogenic real-time assay of the repair of oxidative damage. *ChemBioChem* **2015**, *16* (11), 1637−1646.

(15) Beharry, A. A.; Lacoste, S.; O'Connor, T. R.; Kool, E. T. Fluorescence monitoring of the oxidative repair of DNA alkylation damage by ALKBH3, a prostate cancer marker. *J. Am. Chem. Soc.* **2016**, *138* (11), 3647−3650.

(16) Wilson, J. N.; Cho, Y.; Tan, S.; Cuppoletti, A.; Kool, E. T. Quenching of fluorescent nucleobases by neighboring DNA: the "insulator" concept. *ChemBioChem* **2008**, *9* (2), 279−285.

(17) Fromme, J. C.; Banerjee, A.; Huang, S. J.; Verdine, G. L. Structural basis for removal of adenine mispaired with 8-oxoguaning by MutY adenine DNA glycosylase. *Nature* **2004**, *427*, 652−656.

(18) Francis, A. W.; Helquist, S. A.; Kool, E. T.; David, S. S. Probing the requirements for recognition and catalysis in Fpg and MutY with nonpolar adenine isosteres. *J. Am. Chem. Soc.* **2003**, *125* (52), 16235−16242.

(19) Kimoto, M.; Mitsui, T.; Yokoyama, S.; Hirao, I. A unique fluorescent base analogue for the expansion of the genetic alphabet. *J. Am. Chem. Soc.* **2010**, *132* (14), 4988−4989.

(20) Ichinoe, A.; Behmanesh, M.; Tominaga, Y.; Ushijima, Y.; Hirano, S.; Sakai, Y.; Tsuchimoto, D.; Sakumi, K.; Wake, N.; Nakabeppu, Y. Identification and characterization of two forms of mouse MUTYH proteins encoded by alternatively spliced transcripts. *Nucleic Acids Res.* **2004**, *32* (2), 477−487.

(21) Mitsui, T.; Kimoto, M.; Kawai, R.; Yokoyama, S.; Hirao, I. Characterization of fluorescent, unnatural base pairs. *Tetrahedron* **2007**, *63*, 3528−3537.

(22) Markkanen, E.; Dorn, J.; Hübscher, U. MUTYH DNA glycosylase: the rational for removing undamaged bases from the DNA. *Front. Genet.* **2013**, *4*, 18.

(23) Zambianchi, M.; Maria, F. D.; Cazzato, A.; Gigli, G.; Piacenza, M.; Sala, F. D.; Barbarella, G. Microwave-assisted synthesis of thiophene fluorophores, labeling and multilabeling of monoclonal antibodies, and long lasting staining of fixed cells. *J. Am. Chem. Soc.* **2009**, *131* (31), 10892−10900.

(24) Yang, H.; Clendenin, W. M.; Wong, D.; Demple, B.; Slupska, M. M.; Chiang, J. H.; Miller, J. H. Enhanced activity of adenine-DNA glycosylase (Myh) by apurinic/apyrimidinic endonuclease (Ape1) in mammalian base excision repair of an A/GO mismatch. *Nucleic Acids Res.* **2001**, *29* (3), 743−752.

(25) Porello, S. L.; Leyes, A. E.; David, S. S. Single-turnover and pre-steady-state kinetics of the reaction of the adenine glycosylase MutY with mismatch-containing DNA substrates. *Biochemistry* **1998**, *37* (42), 14756−14764.

(26) Boldogh, I.; Milligan, D.; Lee, M. S.; Bassett, H.; Lloyd, R. S.; McCullough, A. K. hMYH cell cycle-dependent expression, subcellular localization and association with replication foci: evidence

suggesting replication-coupled repair of adenine:8-oxoguaning mispairs. *Nucleic Acids Res.* **2001**, *29* (13), 2802−2809.

(27) Raetz, A. G.; Xie, Y.; Kundu, S.; Brinkmeyer, M. K.; Chang, C.; David, S. S. Cancer-associated variants and a common polymorphism of MUTYH exhibit reduced repair of oxidative DNA damage using a GFP-based assay in mammalian cells. *Carcinogenesis* **2012**, *33* (11), 2301−2309.

(28) Chen, S.-Y.; Chen, H.-H.; Huang, Y.-C.; Liu, S.-P.; Lin, Y.-J.; Lo, S.-F.; Chang, Y.-Y.; Lin, H.-W.; Huang, C.-M.; Tsai, F.-J. Polymorphism and protein expression of MUTYH gene for risk of rheumatoid arthritis. *BMC Musculoskeletal Disord.* **2017**, *18*, 69.