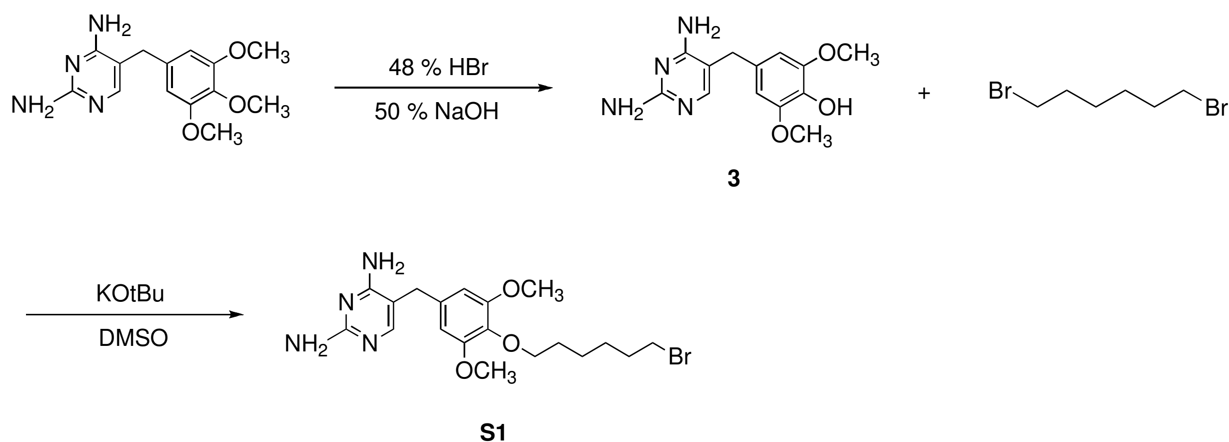
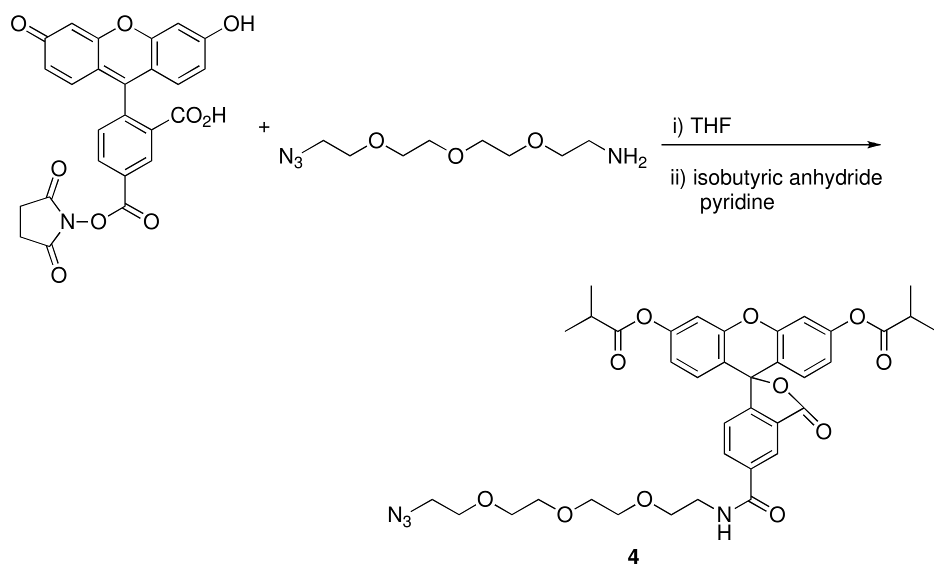
Supplementary Material

Synthetic Chemistry.

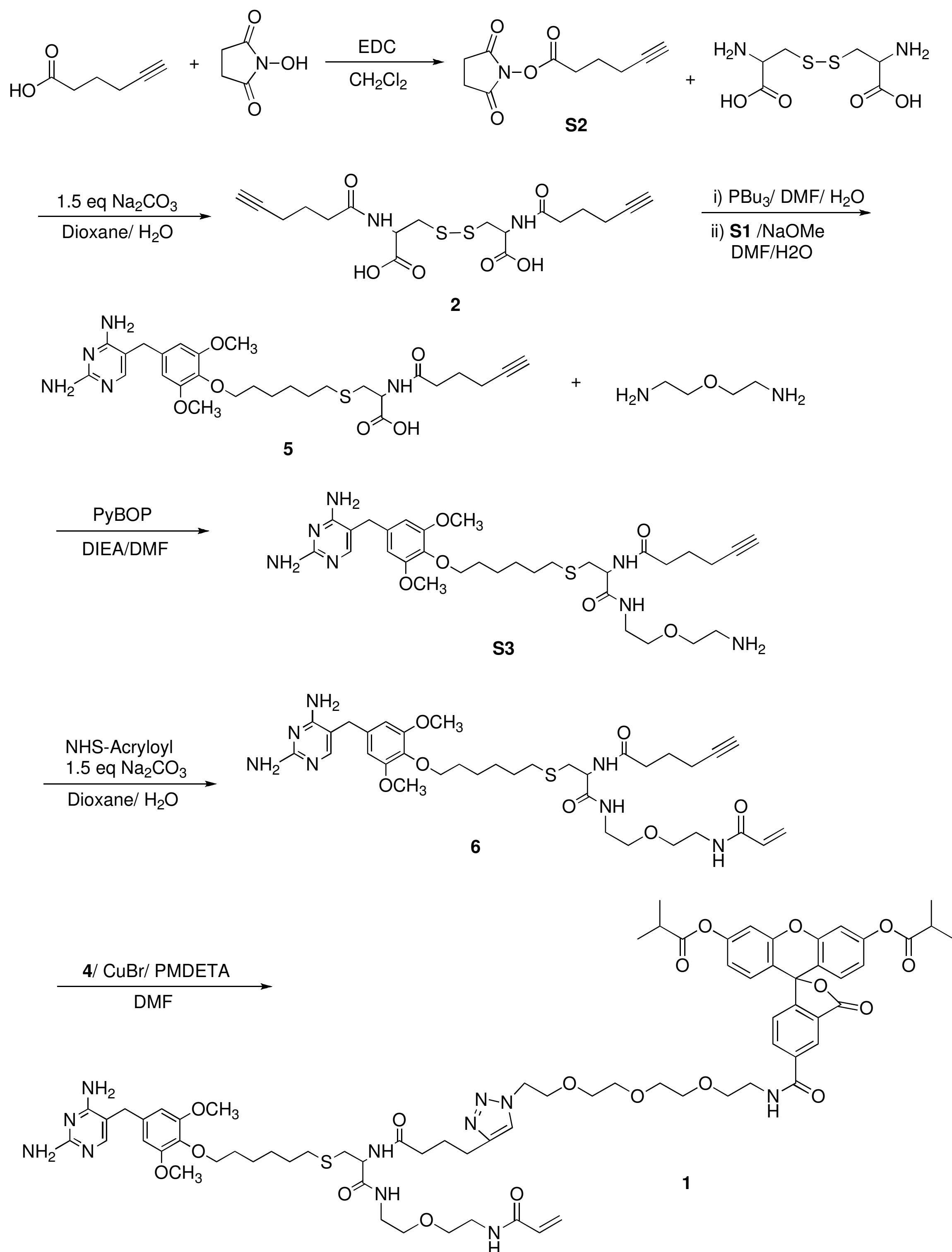
General Methods. Unless otherwise noted, reagents were obtained from commercial suppliers and were used without further purification. Dexmethasone (Dex) was purchased from Sigma, Benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate (PyBOP) was purchased from Advanced ChemTech. Anhydrous *N,N*-dimethylformamide (DMF) and anhydrous methylene chloride (CH_2Cl_2) were from Sure Seal^{TM} bottles purchased from Aldrich. All other reagents for chemical synthesis were purchased from Aldrich. All moisture or oxygen sensitive reactions were performed under a positive pressure of nitrogen in flame- or oven-dried glassware. Nuclear magnetic resonance (NMR) spectra were recorded on a Bruker 500 (500MHz) Bruker 400 (400MHz) or Bruker 300 (300MHz) Fourier Transform (FT) NMR spectrometers at Columbia University, Chemistry Department. ^{1}H NMR spectra are tabulated in the following order: multiplicity (s, singlet; d, doublet; t, triplet; m, multiplet; br, broad), number of protons. Fast Atom Bombardment (FAB) high resolution mass spectra (HRMS) were recorded on a JMS-HX110A mass spectrometer, and low resolution electron spray ionization (ESI) MS were recorded on a JMS-LC mate mass spectrometer.



Scheme S1: Synthesis of compound S1.



Scheme S2: Synthesis of compound 4.



Scheme S3: Longest linear synthetic route for the synthesis of A-TMP-Fl.

Synthesis of TMP-OH (3). A round bottom flask containing 48% aqueous hydrobromic acid (250 mL) was heated to 100˚C. Trimethoprim (25.0 g, 86 mmol) was suspended and stirred for 20 minutes. The flask was removed from the oil bath and 50% sodium hydroxide in water (75 mL) was added. The reaction mixture was then cooled to room temperature and the resulting crystals were filtered and rinsed with cold water. The crystals were then dissolved in hot water (350 mL), neutralized with aqueous NH_4OH and allowed to crystallize. The crystals were filtered, rinsed with cold water, and dried under vacuum to produce 15.9 g (67% yield). ^1H NMR (400 MHz, CD_3OD) δ ppm: 7.18 (s, 1H), 6.53 (s, 2H), 3.81 (s, 6H), 3.62 (s, 2H).

Synthesis of TMP-linker-bromide (S1). Compound **3** (2.0 g, 7.2 mmol) and potassium tert-butoxide (0.98 g, 8.0 mmol) were dried under vacuum for an hour. Next, the compounds were dissolved in dimethyl sulfoxide (DMSO) (46 mL), and 1,6-dibromohexane (5.6 mL, 36 mmol) was added to the solution. The reaction was stirred for 2 hours until complete by TLC. Ethyl acetate (EtOAc) (50 mL) and H_2O (50 mL) were added and the organic and aqueous layers were separated. The aqueous layer was than washed twice with EtOAc (50 mL). The organic layers were combined and washed with H_2O (50 mL). The organic layer was dried with $MgSO_4$ and the solvent was removed *in vacuo*. The crude product was then purified by silica gel flash chromatography (20:1 CH_2Cl_2:MeOH) to give 0.79 g (25% yield) of compound **S1**: R_f = 0.37 in 9:1 CH_2Cl_2:MeOH. ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.50 (s, 1H); 6.51 (s, 2H); 3.90 (t, 2H); 3.77 (s, 6H); 3.46 (s, 2H); 3.44 (t, 2H); 1.86 (t, 2H); 1.69 (t, 2H); 1.50 (t, 4H); MS, m/z 439.12 (MH^+); HRMS, m/z 439.1345 (MH^+), calculated 439.35.

Synthesis of NHS-Yne (S2). 5-hexynoic acid (0.59 mL, 5.4 mmol) and N-hydroxysuccinimide (1.5 g, 13 mmol) were dissolved in CH_2Cl_2 (50 mL). Then, 1-ethyl-3-(3-dimethyllaminopropyl)carbodiimide hydrochloride (EDAC·HCl) (1.5 g, 8.0 mmol) was added and the reaction was stirred under Ar for 15 hours. The reaction mixture was then washed with saturated Na_2CO_3 (250 mL) and the aqueous phase was extracted with anhydrous ether (250 mL). The organic layers were combined and washed with H_2O (250 mL) and brine (250 mL). The organic layer was dried with anhydrous Mg_2SO_4 and the solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica (7:3 Hexanes:EtOAc) to yield compound **S2** (0.98 g, 4.6 mmol, 87%). Rf = 0.2 in 3:7 EtOAc:Hex. ^{1}H NMR (300 MHz, $CDCl_3$) δ ppm: 2.53 (t, 2H); 2.30 (td, 2H); 2.00 (t, 1H); 1.87 (5, 2H).

Synthesis of Cys_2-Yne (2). L-cystine (0.37 g, 1.6 mmol) and Na_2CO_3 (0.49 g, 4.7 mmol) were dissolved in H_2O (12 mL). Compound **S2** (0.98 g, 4.7 mmol) was then dissolved in anhydrous 1,4-dioxane (12 mL) and added dropwise to the solution. The reaction was stirred overnight at room temperature. The solvent was removed and the crude product was purified by column chromatography on silica gel (12:1 CH_2Cl_2:MeOH with 2% AcOH) to yield compound **2** (0.43 g, 1.0 mmol, 64%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 4.62 (d, 2H); 3.35 (dd, 2H); 2.96 (dd, 2H); 2.38 (t, 4H); 2.23 (m, 6H); 1.82 (5, 4H); MS, m/z 429.23 (MH^+), 451.24 (MNa^+), calculated 428.52.

Synthesis of TMP-Yne-COOH (5). Compound **2** (0.30 g, 0.69 mmol) was dissolved in DMF (7.8 mL) and H_2O (0.78 mL) and degassed with Ar for 15 minutes. Then, n-tributylphosphine (0.70 mL, 2.8 mmol) was added to the solution and the reaction was stirred under Ar for 40 minutes. Then the solvent was removed and the crude reaction mixture was dissolved in DMF (0.30 mL). Compound **S1** (0.57 g, 1.3 mmol) was added to the mixture followed by 0.5 M NaOMe in MeOH (20 mL, 10 mmol). The reaction was stirred for 13 hours and the solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (9:1 to 8:2 CH_2Cl_2:MeOH) to yield compound **5** (0.40 g, 0.70 mmol, 54%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.17 (s, 1H); 6.55 (s, 2H); 4.43 (dd, 1H); 3.93 (t, 2H); 3.80 (s, 6H); 3.64 (s, 2H); 3.03 (dd, 1H); 2.82 (m, 1H); 2.54 (t, 2H); 2.36 (m, 2H); 2.21 (m, 3H); 1.80 (m, 2H); 1.66 (5, 2H); 1.56 (5, 2H); 1.43 (m, 4H); MS, m/z 574.3 (MH^+), 596.3 (MNa^+), calculated 573.7.

Synthesis of TMP-Yne-Peg (S3). Compound **5** (0.21 g, 0.37 mmol) and PyBOP (0.49 g, 0.93 mmol) were dried under vacuum for 1 hour. Then they were dissolved in DMF (1.0 mL). 1,5-diamino-3-oxapentane and DIEA were added to the solution and the reaction was stirred at room temperature overnight. The solvent was removed *in vacuo* and the crude product was purified by column chromatography on silica (12:1 CH_2Cl_2:MeOH with 1% Et_3N) to yield compound **S3** (0.17 g, 0.26 mmol, 69%). ^{1}H NMR (400 MHz, CD_3OD) δ ppm: 7.50 (s, 1H); 6.51 (s, 2H); 4.46 (t, 1H); 3.88 (t, 2H); 3.77 (s, 6H); 3.63 (s, 2H); 3.50 (m, 4H); 3.38 (t, 2H); 2.92 (dd, 1H); 2.82 (t, 2H); 2.72 (dd, 1H); 2.57 (t,

2H); 2.36 (t, 2H); 2.25-2.20 (m, 3H); 1.80 (5, 2H); 1.68 (5, 2H); 1.60 (5, 2H); 1.46 (m, 4H); MS, m/z 660.74 (MH^+), calculated 659.84.

Synthesis of A-TMP-Yne (6). Compound **S3** (22 mg, 0.034 mmol) and Na_2CO_3 (5.4 mg, 0.051 mmol) were dissolved in H_2O (0.50 mL). Acrylic acid N-hydroxysuccinimide ester (5.7 mg, 0.034 mmol) was dissolved in anhydrous 1,4-dioxane and added dropwise to the mixture. The reaction was stirred at room temperature overnight and then the solvent was removed *in vacuo*. The crude product was purified by column chromatography on silica gel (18:1 to 9:1 CH_2Cl_2:MeOH) to yield compound **6** (6.4 mg, 8.7 µmol, 27%). ^1H NMR (300 MHz, CD_3OD) δ ppm: 7.43 (s, 1H); 6.52 (s, 2H); 6.34-6.17 (m, 2H); 5.64 (dd, 1H); 4.48 (dd, 1H); 3.89 (t, 2H); 3.78 (s, 6H); 3.63 (s, 2H); 3.52 (5, 4H); 3.43 (t, 2H); 3.36 (t, 2H); 2.93 (dd, 1H); 2.73 (dd, 1H); 2.56 (t, 2H); 2.37 (t, 2H); 2.22 (m, 3H); 1.80 (5, 2H); 1.68 (5, 2H); 1.59 (5, 2H); 1.46 (m, 4H); MS, m/z 714.48 (MH^+); HRMS, m/z 714.3664 (MH^+), calculated 713.89.

Synthesis of the isobutryl protected azido-fluorescein (4). 11-azido-3,6,9-trioxaundecan-1-amine (8.0 µL, 40 mmol) was added to a solution of 6-carboxyfluorescein *N*-succinimidyl ester (6.0 mg, 0.013 mmol) was dissolved in THF (0.20 mL). The reaction was stirred under Ar for 15 hours and then concentrated *in vacuo*. Then the crude product was redissolved in pyridine (0.40 mL). Isobutyric anhydride (0.62 mL, 3.7 mmol) was added and the mixture was stirred at room temperature for 2 hours, during which the color and fluorescence disappeared. Water (20 µL) was added to the mixture and incubated for 5 minutes. Then the solvent was removed *in vacuo* and the crude product was evaporated twice with toluene. The crude product was purified by column chromatography on silica gel (80:1 to 40:1 CH_2Cl_2:MeOH) to yield compound **4** (1.9 mg, 2.7 µmol, 21%). ^1H NMR (300 MHz, CD_3OD) δ ppm: 8.48 (s, 1H); 8.23 (d, 1H); 7.36 (d, 1H); 6.89 (s, 4H); 3.71-3.60 (m, 14H); 2.83 (7, 2H); 1.29 (d, 12H).

Synthesis of A-TMP-Fl (1). Compound **4** (7.0 mg, 9.8 µmol) and compound **6** (6.3 mg, 9.8 µmol) were transferred to the same vial and dried under vacuum for an hour. DMF (100 µL) and *N*,*N*,*N'*,*N'*,*N*-pentamethyldiethylenetriamine (PMDETA) (4.0 µL) were degassed for 15 minutes with Ar and added to the reaction vial. Copper (I) bromide (CuBr) (0.70 mg, 4.9 µmol) was added and the solution was degassed via three freeze-pump-thaw cycles. The reaction was stirred for 24 hours and then purified by HPLC to yield compound **1** (1.1 mg, 0.80 µmol, 8%). ^1H NMR (500 MHz, CD_3OD) δ ppm: 8.47 (s, 1H); 8.23 (d, 1H); 8.15 (bs, 1H); 7.37 (t, 1H); 7.22 (s, 1H); 7.17 (d, 2H); 6.88 (d, 4H); 6.54 (d, 2H); 6.25 (m, 2H); 5.62 (d, 1H); 4.50 (t, 1H), 3.89 (m, 2H), 3.77 (s, 6H), 3.69-3.50 (m, 18H), 3.40 (m, 4H), 3.18 (m, 2H), 2.86 (m, 3H), 2.72 (m, 2H), 2.58 (t, 1H), 2.54 (m, 2H), 2.45 (m, 1H), 2.30 (m, 1H), 1.96 (m, 2H), 1.64 (m, 2H), 1.56 (m, 2H), 1.41 (m, 4H), 1.28 (d, 12H); MS, m/z 1428.40 (MH^+), 1454.12 (MNa^+), 1471.75 (MK^+), calculated 1430.62.

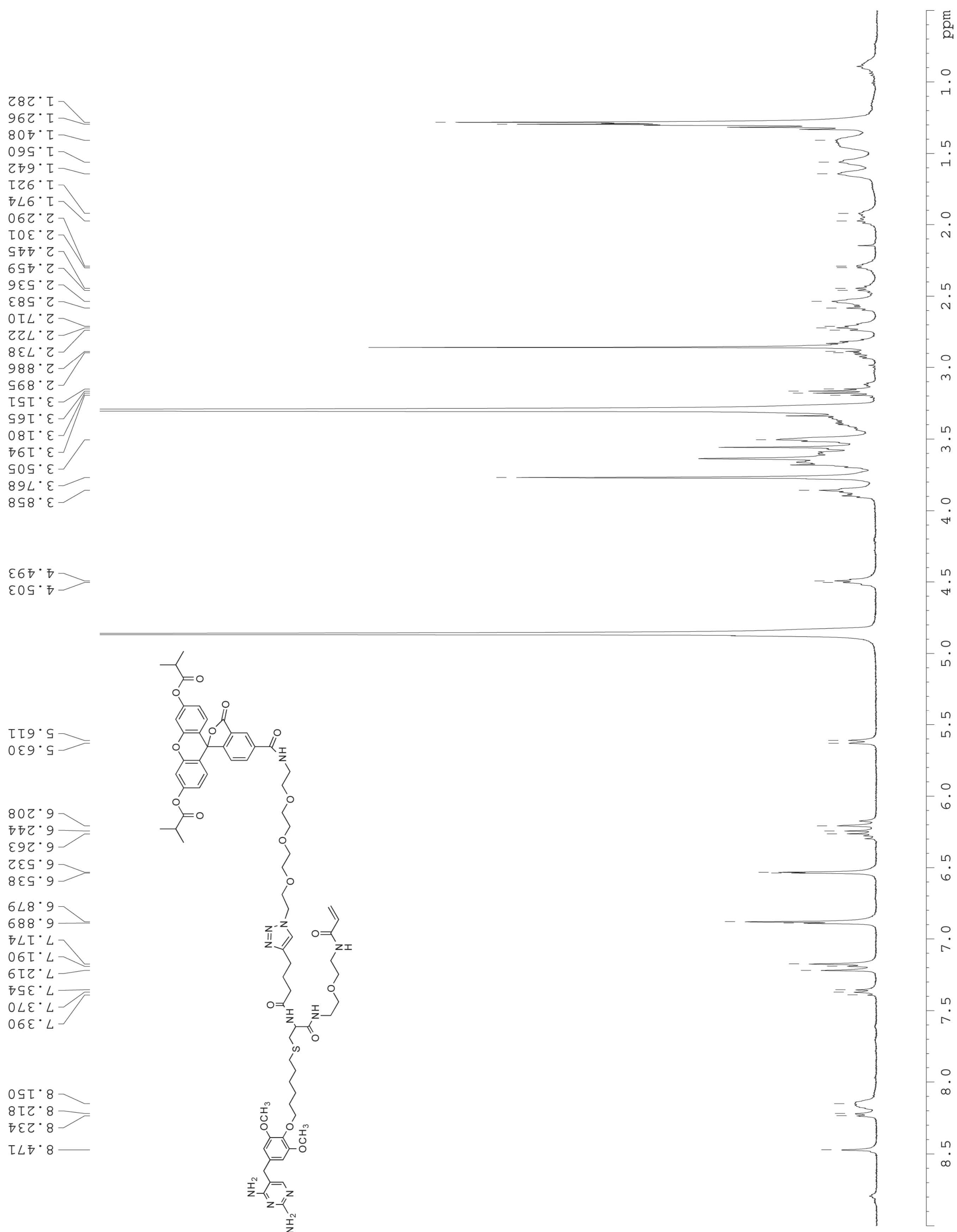


Figure S1: ^{1}H-NMR of A-TMP-Fl (**1**) (500 MHz, CH_3OD).

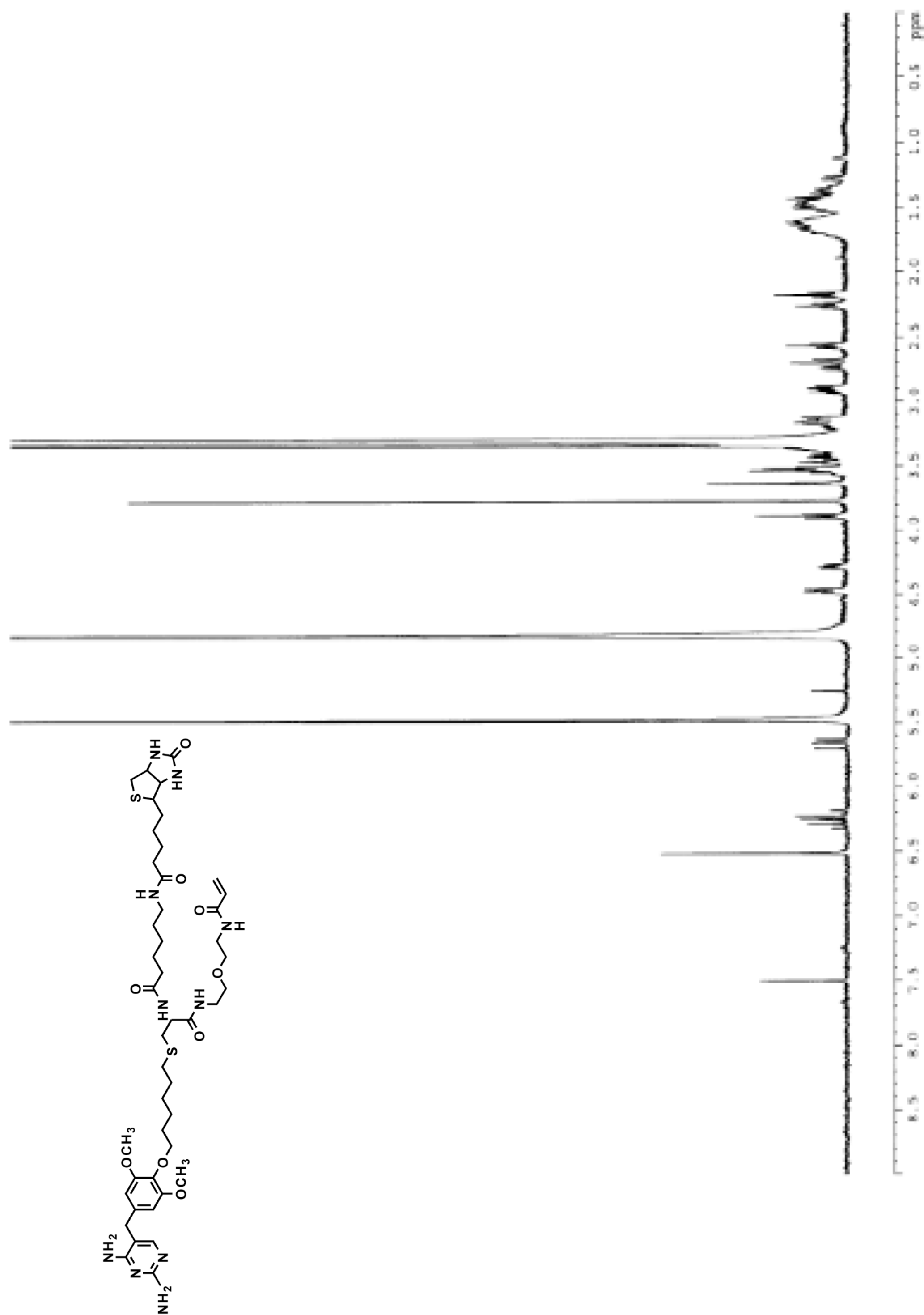


Figure S2: ^{1}H-NMR of A-TMP-Fl (400 MHz, CH_3OD).

Experimental Methods for In-Gel Fluorescence Scanning

Nucleofection. NIH Swiss 3T3 fibroblast cells were washed once with PBS and harvested with trypsin/EDTA. Trypsinization was stopped by addition of supplemented Dulbecco's modified Eagle medium (DMEM) and an aliquot of the cell suspension was counted to determine the cell density. Subsequently, $1x10^6$ cells were transferred to a conical tube and centrifuged for 5 minutes at 1000 rpm. Then, the media was removed and the pellet was resuspended Nucleofector Solution R to a final concentration of $1x10^6$ cells per 100 µL. The cell suspension was mixed with 5 µg of DNA and the mixture was transferred to an AMAXA cuvette and program U-30 was run. Then, 500 µL of pre-warmed culture media was added with the plastic pipet and the cells were transferred to a 10 cm tissue culture treated petri dish containing 15 mL of pre-warmed culture media. For each 10 cm dish, cells were added from two separate nucleofections with the same DNA construct. The media was replaced with fresh media 24 hours after transfection.

In-Gel Fluorescence Scanning. Cells were incubated at 37˚C for 48 hours after transfection. Then, the cells were washed once with PBS and 4 mL of supplemented DMEM was added to each dish. For designated dishes, 4 mL of supplemented DMEM was added that contained 1 µM of A-TMP-Fl. The cells were then incubated for 3 hours at 37˚C, washed twice with PBS and harvested with trypsin/EDTA. Trypsinization was stopped by addition of DMEM and the cell density was determined. The cells were then pelleted by centrifugation at 1000 rpm for 4 minutes. The supernatant was removed, and the pellet was washed with 1 mL of PBS per $1x10^6$ cells and repelleted. Again, the supernatant was removed and the pellet was resuspended in lysis buffer to a concentration of $1x10^6$ cells in 12.5 µL of lysis buffer and lysed on ice for 20 minutes. The lysate was then centrifuged at 13000 rpm for 10 minutes and the supernatant was mixed with loading buffer and heated to 95˚C for five minutes. Samples from the *in vivo* covalent labeling experiments were run on Criterion 10.5-14% Tris-HCl gels (BioRad) for 1.5 hours at 150 V. The BenchMark™ Fluorescent Protein Standard from Invitrogen (Catalog No: LC5928) was used to determine the protein molecular weights. The proteins were detected by in-gel fluorescence scanning using a Typhoon™ Trio scanner. The gel was analyzed for green and red fluorescence by scanning with the 488 and 532 nm lasers, respectively, and an emission filter of 520 (BP 40) and 580 (BP 30), respectively. The PMT value was adjusted for each channel and a high-resolution scan was collected at 100 µm resolution.

Experimental Methods for Vector Construction

Site-Directed Mutagenesis. Genes encoding eDHFR variants were produced using Strategene's QuikChange Mutagenesis Kit. The parent vector was the previously published p1008 (eDHFR in pAED4) (*38*). Three successive rounds of mutagenesis yielded p2250, which is an eDHFR variant with three substitutions: L28C, C85S, and C152S. Primers for mutagenesis: 5'-GCC ATC GCG GCG TCC GGT GAC GTA CCA G-3' and 5'-CTG GTA CGT CAC CGG ACG CCG CGA TGG C-3' (C85S, p2246); 5'-GAA CTC TCA CAG CTA TAG CTT TGA GAT TCT GGA GC-3' and 5'-GCT CCA GAA TCT CAA AGC TAT AGC TGT GAG AGT TC-3' (C85S and C152S, p2247); 5'-GGA ACC TGC CTG CCG ATT GCG CAT GGT TTA AAC GCA AC-3' and 5'-GT TGC GTT TAA ACC ATG CGC CAG GCA GGT TCC-3' (C85S, C152S, and L28C, p2250).

Construction of eDHFR:L28C-RFP-NLS. eDHFR:L28C-RFP-NLS: The gene encoding eDHFR:L28C was subcloned into pEGFP-NLS (Clontech). A fragment encoding eDHFR:L28C with an N-terminal Kozak sequence and valine in the second position was prepared with two rounds of PCR using pAED4 eDHFR:L28C as template and the primers 5'-GCA TAC GTC GCT AGC GCT ACC GGT CGC CAC CAT GGT GCA TCA CC-3' (2nd round, NheI, coding strand) and 5'-CAC CAT GGT GCA TCA CCA TCA CCA TCA CAT CAG TCT GAT TGC GGC-3' (1st round, coding strand) and 5'-GCA TAC GTC CTC GAG ATC TGA GTC CGG ACC GCC GCT CCA GAA TC-3' (XhoI, noncoding

strand). The fragment was inserted between the NheI and XhoI sites in pEGFP-NLS to give eDHFR:L28C-NLS (p2275). The gene encoding RFP was amplified using PCR from the pDsRed-Monomer-N1 vector (Clontech) with primers 5'-GCA TAC GTC CTC GAG TGA TGG ACA ACA CCG AGG A-3' (XhoI, coding strand) and 5'-GCA TAC GTC TCG AGA ACC AGA ACC ACC AGA ACC CTG GGA GCC GGA GTG G-3' (XhoI, noncoding strand). The fragment was then inserted in the XhoI site of eDHFR:L28C-NLS to give eDHFR:L28C-RFP-NLS (p2355). The resulting coding region was sequenced in full.

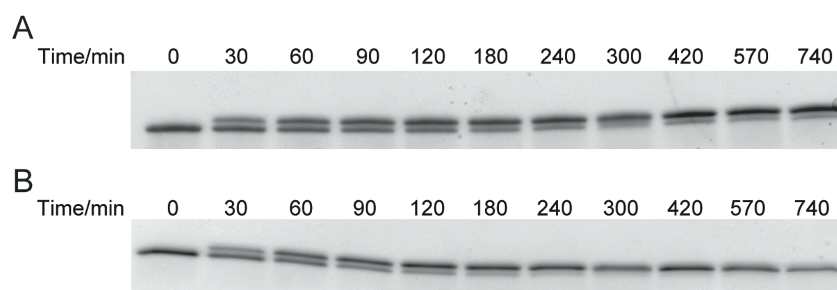


Figure S3: Determination of the rate of covalent labeling *in vitro* for different reaction conditions. Additional kinetic runs were preformed using the same experimental method that was described in the paper. The rate was determined for the alkylation reaction when stoichiometric amounts of eDHFR:L28C and A-TMP-B were incubated. A-B) Stoichiometric amounts of purified eDHFR:L28C incubated with A-TMP-B in PBS containing 1 mM glutathione at 37˚C. A) Both reactants at a 5 µM concentration. B) Both reactants at a 1 µM concentration. In both cases, the time required for 50% reaction was determined to be 50 minutes from the pseudo first-order rate constant for the appearance of the alkylated product. This result shows that the rate is the same even when stoichiometric amounts of reactants are used, and even at concentrations as low as 1 µM.

COPASI Model

A. Enzyme/Substrate
1. $E + S \leftrightarrow C$
2. $C \rightarrow P$

B. eDHFR:L28C/A-TMP-tag
1. $D + T \leftrightarrow C$
2. $C \rightarrow P$

The enzyme chosen was a mutant haloalkane dehalogenase with higher catalytic activity [1].

The starting concentrations of C and P were set to 0. E, S, D and T varied as listed below.

Kinetic rates:

A1: $k_{on} = 960\ M^{-1}s^{-1}$; $k_{off} = 9.6\ s^{-1}$
A2: $K_M = 4.3\ \mu M$; $V_{max} = k_{cat}$*[enzyme]; $k_{cat} = 2.8\ s^{-1}$
B1: $k_{on} = 1*10^7\ M^{-1}s^{-1}$; $k_{off} = 0.009\ s^{-1}$
B2: $k_{app} = 0.000231\ s^{-1}$

Table I: Time Required for 50% Labeling By Enzymatic Modification.

Enzyme (µM)	Substrate (µM)	Time (s)	Time (min)	Time (hr)
5	5	823.2	13.72	0.23
1	1	16410	273.50	4.56
0.1	0.1	1547000	25783.33	429.72
0.01	0.01	153400000	2556666.67	42611.11
0.001	0.001	15400000000	256666666.67	4277777.78

Table II: Time Required for 95% Labeling By Enzymatic Modification.

Enzyme (µM)	Substrate (µM)	Time (s)	Time (min)	Time (hr)
5	5	15632	260.53	4.34
1	1	311600	5193.33	86.56
0.1	0.1	29370000	489500.00	8158.33
0.01	0.01	2920000000	48666666.67	811111.11
0.001	0.001	292000000000	4866666666.67	81111111.11

Table III: Time Required for 50% Labeling By A-TMP-tag.

eDHFR:L28C (µM)	A-TMP (µM)	Time (s)	Time (min)	Time (hr)
5	5	3050	50.83	0.85
1	1	3111	51.85	0.86
0.1	0.1	3364	56.07	0.93
0.01	0.01	4304	71.73	1.20
0.001	0.001	8937	148.95	2.48

Table IV: Time Required for 95% Labeling By A-TMP-tag.

eDHFR:L28C (µM)	A-TMP (µM)	Time (s)	Time (min)	Time (hr)
5	5	13380	223.00	3.72
1	1	13910	231.83	3.86
0.1	0.1	16230	270.50	4.51
0.01	0.01	26610	443.50	7.39
0.001	0.001	99088	1651.47	27.52

Quantification of the Relative Band Intensities (Figure 4, panel b)

There are a few minor higher and lower molecular weight bands in the lysate of the transfected cells. In order to determine the relative intensities of these bands, the gel was analyzed in ImageQuant TL. The lanes were created manually, but then the program performed the background subtraction and band detection automatically.

Bands were only detected above the background in Lane 1, the lysate of the transfected cells, and Lane 4, the GFP control. Below is the table of the measurements of the bands detected by ImageQuant TL for Lane 1. The relative band intensity was quantified for four bands identified as above the background. The table shows that the desired labeled product accounts for 49% of the fluorescence.

Table V: Quantification of Relative Band Intensitity.

Band No.	Approx. MW	Volume	Vol+BkGnd	Band % (Norm)
1	120	7436.90	57936	19.32
2	90	3603.73	53541	9.36
3	52	18863.75	77129	49.01
4	30	8588.82	71547	22.31

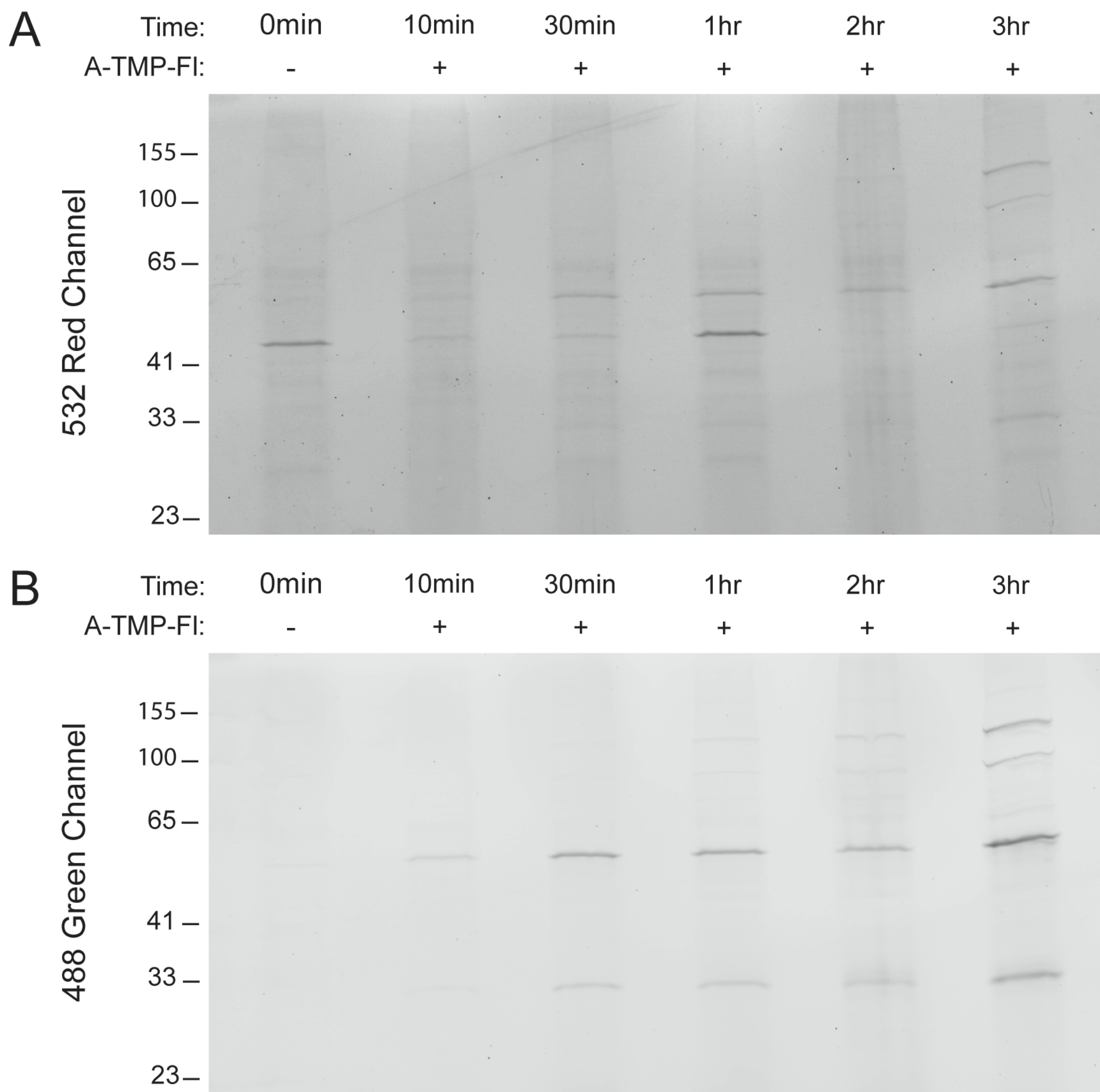


Figure S5: Time course for *in vivo* covalent labeling. To determine the extent of and time required for covalent labeling *in vivo*, NIH 3T3 fibroblasts were nucleofected with vector DNA encoding a nucleus-targeted eDHFR:L28C-RFP fusion protein. Cells were incubated with or without A-TMP-Fl at 37˚C for various lengths of times, washed twice, trypsinized, pelleted and lysed. (A-B) In-gel fluorescence to determine approximate kinetics of *in vivo* labeling. Lane 1 corresponds to transfected cells incubated without A-TMP-Fl. Lanes 2-6 correspond to transfected cells incubated with 1 µM A-TMP-Fl for various times: Lane 2, 10 minutes; Lane 3, 30 minutes; Lane 4, 1 hour; Lane 5, 2 hours; and Lane 6, 3 hours. The lysates were then analyzed by SDS-Page and in-gel fluorescence scanning. (A) In-gel fluorescence scanning to detect red fluorescence from 532 nm excitation. (B) In-gel fluorescence scanning to detect green fluorescence from 488 nm excitation. This gel shows that after 2 hours covalent labeling *in vivo* is complete with >95% of the protein being covalently labeled.

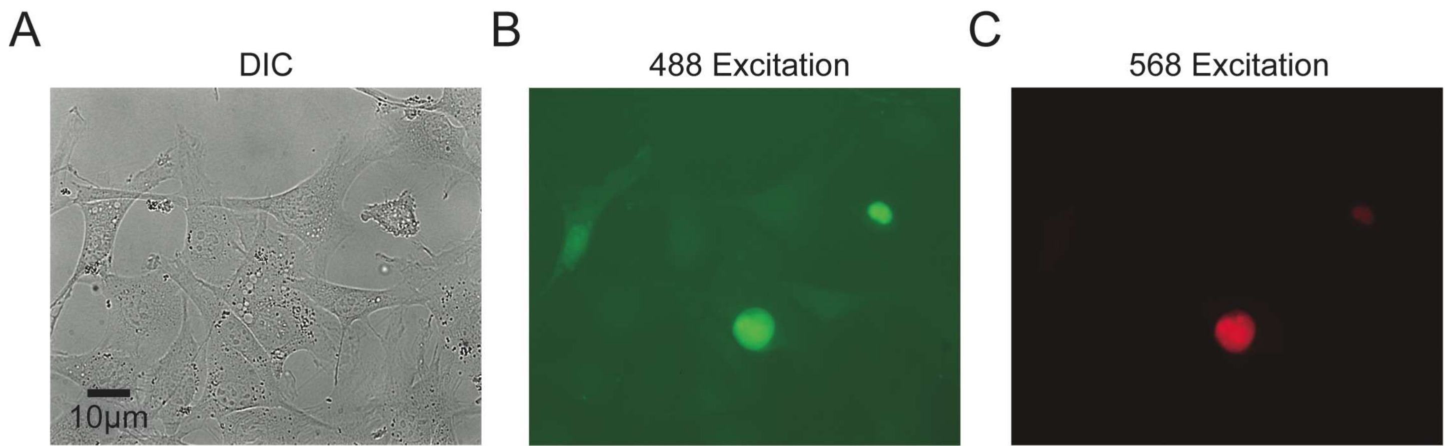


Figure S6: Live cell imaging using A-TMP-Fl. Fibroblasts were transiently transfected with DNA encoding a nucleus-targeted eDHFR:L28C-RFP fusion protein. After 24 hours, the cells were incubated in media containing 1 μM A-TMP-Fl for ten minutes, washed twice with PBS and then imaged one hour later. The image was taken using a 60X 1.4na objective to capture a large number of cells to ensure that both transfected and untransfected cells were in the field of view. The image shows that only a low level of A-TMP-Fl enters untransfected cells, allowing for a very high signal-to-noise ratio in transfected cells.

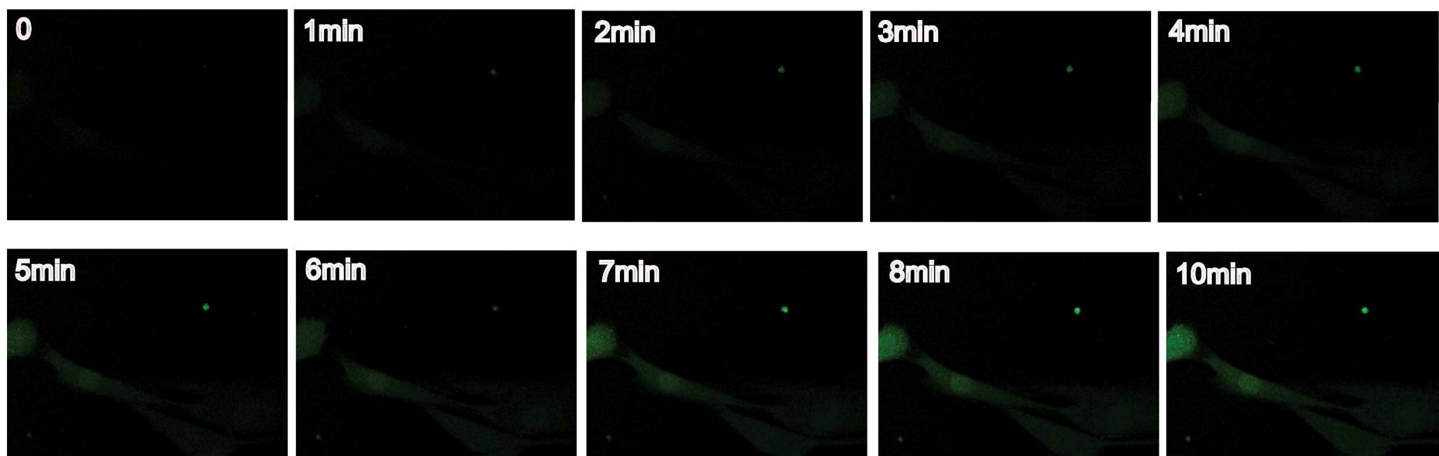


Figure S7: Time required for A-TMP-Fl to enter living cells. NIH 3T3 fibroblasts were transiently transfected with a nucleus-targeted eDHFR:L28C-RFP fusion protein. An image was taken before A-TMP-Fl was added to the media. Then, the media was exchanged for Ringers solutions with 10% serum containing 1 µM A-TMP-Fl. Images were acquired every minute for 10 minutes using an Olympus FVX confocal. Above, images of only the fluorescein channel show a time course of incubation from t=0 to 10 minutes. By ten minutes, enough dye has loaded into the cell to provide sufficient staining of the nucleus. Washing the cells and incubating them in dye free media for an hour, as was done for the images in the manuscript, dramatically reduced background cytostolic fluorescence from free A-TMP-Fl.

1. Schanstra, J.P., et al., *Kinetic Characterization and X-ray Structure of a Mutant of Haloalkane Dehalogenase with Higher Catalytic Activity and Modified Substrate Range†,‡*. Biochemistry, 1996. **35**(40): p. 13186-13195.