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## RESEARCH ARTICLE

## CHEMICAL BIOLOGY

# Redox-based reagents for chemoselective methionine bioconjugation

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Cysteine can be specifically functionalized by a myriad of acid-base conjugation strategies for applications ranging from probing protein function to antibody-drug conjugates and proteomics. In contrast, selective ligation to the other sulfur-containing amino acid, methionine, has been precluded by its intrinsically weaker nucleophilicity. Here, we report a strategy for chemoselective methionine bioconjugation through redox reactivity, using oxaziridine-based reagents to achieve highly selective, rapid, and robust methionine labeling under a range of biocompatible reaction conditions. We highlight the broad utility of this conjugation method to enable precise addition of payloads to proteins, synthesis of antibody-drug conjugates, and identification of hyperreactive methionine residues in whole proteomes.

Sulfur occupies a privileged place in biology owing to its versatile and unique chemistry (1). Although cysteine and methionine are the only two sulfur-containing proteinogenic amino acids, the sulfur center plays a diverse array of critical roles spanning catalysis to metal binding to redox regulation and other posttranslational modifications (2–4). In this context, selective protein conjugation methods based on cysteine modification have enabled a broad range of fundamental and applied advances (5, 6), from probes of protein function (4, 7–9) to synthesis of covalent small-molecule inhibitors (10, 11) and antibody-drug conjugates (12) to activity- and reactivity-based protein profiling for functional cysteine identification (7, 13, 14) and inhibitor development (15) (Fig. 1A). Cysteine bioconjugation strategies typically exploit the intrinsically high nucleophilicity of the thiol/thiolate side chain, including elegant methods based on electrophilic warheads such as maleimides and alkyl and aryl halides (16, 17), transition metal-mediated bioconjugation (18), and cysteine-to-dehydroalanine conversion (16, 19, 20).

In contrast to the substantial body of literature on cysteine bioconjugation, analogous methods for methionine labeling under physiological conditions remain largely underdeveloped. Despite a number of compelling motivations for this pursuit, previous methods have generally employed highly electrophilic reagents to convert methionine to sulfonium salts (21, 22) at low pH. Methionine is among the most hydrophobic and the second rarest amino acid in vertebrates, and taken together with the fact that the majority of methionine residues are buried within interior protein cores (1), surface-accessible methionines are limited and offer a potentially valuable handle for highly selective protein modification using naturally occurring amino acid side chains. In addition, posttranslational modifications of methionine, including by oxidation and/or metal binding (2, 23), are emerging as critical nodes in signaling pathways that control function at the cell and organism level. For example, reversible oxidation of specific methionine residues within actin can control its assembly and disassembly to serve as a navigational signal (24, 25), and the antioxidant function of methionine sulfoxide reductase has been linked to regulation of life span (26). In addition, recent work suggests that methionine oxidation can also increase binding interactions with aromatic residues within proteins (27).

A major chemical challenge in developing a selective methionine modification reaction under pH-neutral physiological conditions is its relatively weak nucleophilicity, which precludes the traditional approach of identifying an appropriate methionine-specific electrophilic partner for its acid-base bioconjugation in the presence of competing, more nucleophilic amino acids such as cysteine, lysine, tyrosine, or serine (16, 22, 28)

(Fig. 1A). As such, we sought to pursue redox reactivity as an alternative strategy for methionine ligation and now report a method, termed redox-activated chemical tagging (ReACT), that enables chemoselective methionine bioconjugation (Fig. 1A) in proteins and proteomes.

## Development of ReACT for chemoselective methionine bioconjugation

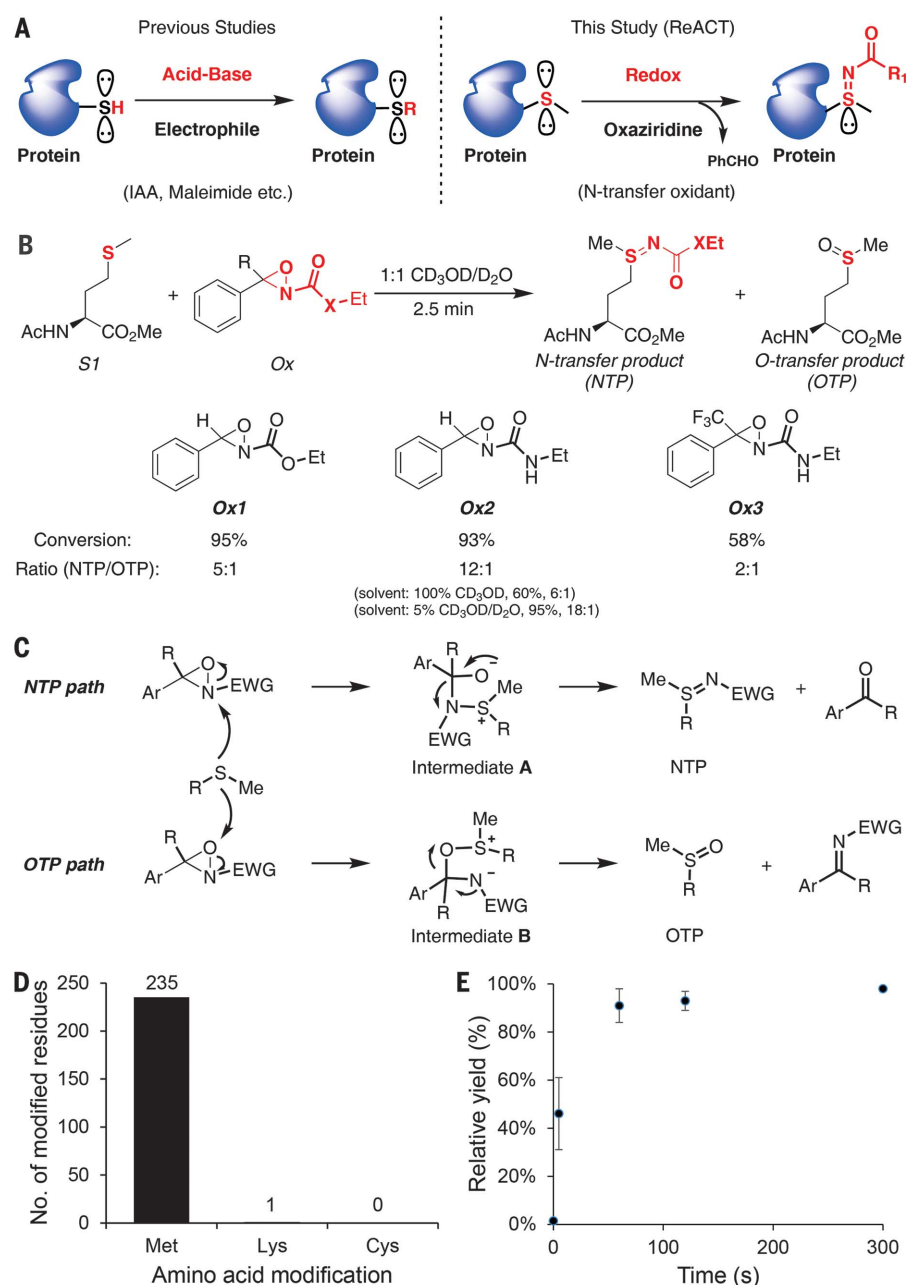
Inspired by observations of facile autoxidation of methionine residues to methionine sulfoxides during mass spectrometry analyses, we reasoned that an oxidative sulfur imidation reaction (29) might serve as an attractive starting point for the ReACT strategy, owing to the flexibility of introducing various functionalities on the nitrogen pendant (Fig. 1A and fig. S1). We initiated our study by screening a variety of sulfur imidation reactions with methionine derivative S1 as a model substrate in 1:1 CD<sub>3</sub>OD/D<sub>2</sub>O solvent using proton nuclear magnetic resonance (<sup>1</sup>H NMR) analysis of substrate conversion and reaction selectivity between the desired N-transfer product (NTP, sulfimide) and unwanted O-transfer product (OTP, sulfoxide) (Fig. 1B). Surveys of various transition metal-catalyzed sulfur imidation reactions were unfruitful, resulting in either no conversion or formation of sulfoxide as the only product (fig. S1). However, a strain-driven sulfur imidation of methionine using oxaziridine 1 (Ox1) as the sulfur imidation reagent afforded 95% conversion of S1 within 2.5 min without additional catalyst with a NTP:OTP ratio of 5:1 (Fig. 1B). On the basis of previous reports that oxaziridines substituted with an electron-withdrawing group (EWG) favored formation of the OTP (30), a carbamate substituted with a weak EWG was initially examined (Fig. 1C). From this starting point, altering the linkage of the probe from carbamate to a weaker electron withdrawing urea (Ox2) resulted in enhanced selectivity (NTP:OTP = 12:1) with comparable conversion. Further attempts to tune electronic effects by substitution of the benzylic hydrogen of Ox2 with an electron withdrawing CF<sub>3</sub> group (Ox3) resulted in much lower selectivity (NTP:OTP = 2:1) and reaction conversion (58%), likely as a result of increased steric hindrance. We observed a marked improvement in NTP:OTP selectivity from 6:1 to 18:1 by increasing the percentage of water in the solvent medium from 0 to 95% (Fig. 1B). In accord with previously posited hypotheses (30, 31), this improvement likely results from increased stabilization of the transition state leading to intermediate A, which should be improved by solvation and hydrogen bonding to the developing alkoxy anion (Fig. 1C). Together, these data presage the utility of this ligation reaction in biological environments.

We next evaluated the reactivity of oxaziridine probes with other biologically relevant amino acid competitors. In all cases, we did not observe any conjugation products with any of the other amino acids tested; only methionine gave a ligated product with the ReACT reagent. Cysteine as well as selenocysteine were oxidized to their cystine forms, with no NTP formation observed (fig. S2), attesting to the high selectivity of the ReACT

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**Fig. 1. The ReACT strategy for chemoselective methionine bioconjugation.**

(A) (Left) Acid-base conjugation strategies for cysteine-based protein functionalization. Iodoacetamide (IAA) and maleimide reagents are representative electrophiles for selective cysteine bioconjugation. (Right) ReACT strategies for methionine-based protein functionalization. Oxaziridine (Ox) compounds serve as oxidant-mediated reagents for direct functionalization by converting methionine to the corresponding sulfimide conjugation product. During this redox process, the Ox ReACT reagents are reduced to benzaldehyde. (B) Model redox conjugation reaction with 25  $\mu$ M of *N*-acetyl-L-methionine methyl ester (S1) and 275  $\mu$ M of various oxaziridine compounds as substrates in cosolvent ( $\text{CD}_3\text{OD}/\text{D}_2\text{O} = 1:1$ ). The reactions were monitored by detecting the chemical shift of the methionine methyl group with  $^1\text{H}$  NMR (fig. S1). The reaction time was 10 min in 100%  $\text{CD}_3\text{OD}$  solution due to slow reaction rate and 20 min in 5%  $\text{CD}_3\text{OD}/\text{D}_2\text{O}$  solution due to poor solubility of substrate in aqueous solution. (C) The proposed reaction mechanism between methionine and oxaziridine compound proceeds by nucleophilic attack of sulfide at N atom or O atom of oxaziridine ring, followed by N–O bond cleavage to generate reaction intermediate A or B, respectively. The NTP or OTP is generated, along with the corresponding aldehyde or imine as side product, through an intramolecular rearrangement. (D) Number of unique ReACT-sensitive Met, Lys, and Cys residues detected in HeLa cell lysates when treated with 1 mM Ox4 for 10 min. (E) Yield of conjugation reaction was performed with 15  $\mu$ M of BSA carrying four methionines per protein and 100  $\mu$ M Ox4 at the indicated time point as measured by in-gel fluorescence imaging. Error bars, mean  $\pm$  SD from three independent experiments. Representative fluorescent gel is shown in fig. S4.



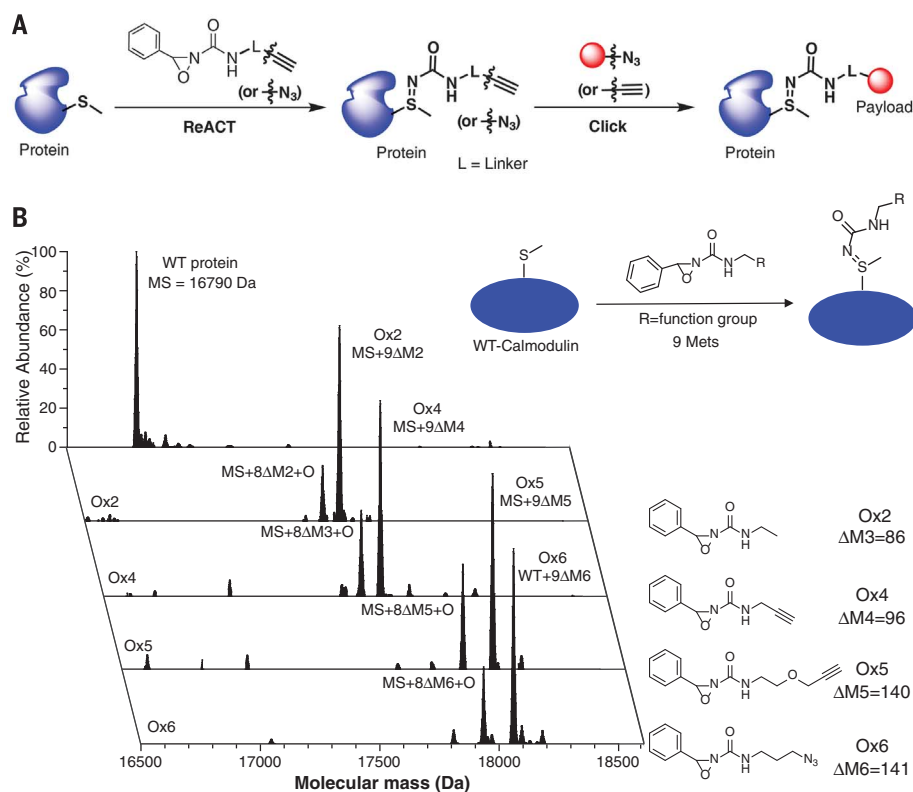
reagent for methionine functionalization over its sulfur congener. As a further demonstration of the high selectivity of ReACT for methionine conjugation, we next identified sites of probe labeling within a whole proteome using liquid chromatography-tandem mass spectrometry (LC-MS/MS) analysis. HeLa cell lysates were treated with Ox4, trypsin digested, and then analyzed by LC-MS/MS for probe modification on all nucleophilic amino acids using the X!Tandem program (32). We observed labeling of 235 methionine residues and a single lysine residue, with no other modifications detected on cysteine side chains or other nucleophilic amino acids (Fig. 1D and Data S1). These experiments demonstrate the fast kinetics of the ReACT strategy as well as near-perfect selectivity for methionine residues from the single-protein

to whole-proteome level under mild biocompatible conditions. Finally, we tested the chemical stability of the sulfimide methionine conjugation product, finding that this linkage showed reasonable stability to acidic and basic conditions, as well as treatment with a strong protein disulfide reducing agent such as tris(2-carboxyethyl)phosphine (TCEP) (fig. S3). The product is stable to exposure to elevated temperatures (80°C) for short times (1 hour), but prolonged exposure (18 hours) can lead to substantial decomposition (fig. S3).

#### ReACT-based bioconjugation to methionines in model protein substrates

We then evaluated ReACT as a method for site-selective methionine conjugation of proteins. Starting with bovine serum albumin (BSA) as a model

protein using a two-step labeling protocol, BSA at a concentration of 15  $\mu$ M was first treated with 100  $\mu$ M oxaziridine probe Ox4 bearing a bio-orthogonal alkyne group and then subsequently coupled to Cy3-azide through a copper-catalyzed azide-alkyne cycloaddition (CuAAC) reaction. The resulting redox conjugation yield to BSA was analyzed by in-gel fluorescence imaging. ReACT proceeds rapidly and can be completed with a yield >95% within 1 to 2 min, with 50% of labeling occurring within the first 5 s after the addition of Ox4 to the protein under standard reaction conditions (Fig. 1E and fig. S4). Moreover, the measured second-order rate constant for reaction of methionine (at 5- to 40-fold excess) with Ox2 in phosphate-buffered saline is  $18.0 \pm 0.6 \text{ M}^{-1} \text{ s}^{-1}$ , which is comparable to what is observed for the



**Fig. 2. The ReACT strategy for protein functionalization.** (A) General two-step procedure for methionine-specific protein functionalization is a combination of ReACT and click reactions. Various payloads (red sphere) can be installed through methionine conjugation at a directed position on a given protein. (B) Redox conjugation of a CaM model protein (100  $\mu$ M) with various Ox compounds (1 mM). The chemical structures of oxaziridine probes are shown with molecular weight changes ( $\Delta$ M) listed for the corresponding modifications. The deconvoluted MS data of full protein peaks are plotted in the same figure. The major peaks correspond to CaM protein carrying nine sulfimide modifications ( $\Delta$ M). For Ox2-labeled protein: expected mass 17,564 Da, found 17,565 Da; Ox4-labeled protein: expected mass 17,654 Da, found 17,654 Da; Ox5-labeled protein: expected mass 18,050 Da, found 18,051 Da; Ox6-labeled protein: expected mass 18,059 Da, found 18,060 Da. The minor peaks correspond to CaM protein bearing eight sulfimide modifications ( $\Delta$ M) and one sulfoxide modification (O).

CuAAC reaction (fig. S5). We then moved on to apply ReACT to modification of calmodulin (CaM) as a model protein with redox-sensitive methionines (33). CaM carries nine redox-active methionine residues, and upon pretreatment with increasing concentrations of hydrogen peroxide, we observed the expected dose-dependent decrease in Ox4 labeling as these residues were oxidized from methionine to methionine sulfoxide, the latter of which is insensitive to ReACT (fig. S6). Only methionine residues were identified carrying the desired modification by LC-MS/MS analysis of Ox4-labeled calmodulin, with no probe-generated conjugation modification observed on any other amino acids (fig. S6). The results presage the potential application of ReACT to probe redox-sensitive methionines by distinguishing them from their oxidized forms.

With these data in hand, we envisioned that ReACT could enable installation of various payloads onto a protein of interest at defined methionine sites, serving as a method for functionalization using naturally occurring amino acids (Fig. 2A). To this end, we evaluated the reactivity of various

alkyne- and azide-containing oxaziridine probes (1 mM) with a 100- $\mu$ M CaM model protein (Fig. 2B). LC-MS/MS results showed that ReACT enabled near quantitative installation of these bioorthogonal handles on all nine native methionine residues within 10 min of labeling time at room temperature, with a 25:1 selectivity over the only other observed minor CaM product bearing eight sulfimide modifications (NTP) and one sulfoxide modification (OTP) (Fig. 2B). Further functionalizations with biotin, fluorophore, and polyethylene glycol (PEG) payloads proceeded smoothly (fig. S7). The high methionine reactivity and specificity of ReACT coupled with the ready availability of click reaction partners provides a straightforward method for precise protein functionalization based on this naturally occurring amino acid (Fig. 2A).

### Application of ReACT to antibody bioconjugation

To showcase the utility of ReACT in a pilot biological application, we turned our attention to the synthesis of antibody-drug conjugates (ADCs),

using the antibody fragment to green fluorescent protein (GFP-Fab) as a starting model. We noted that although the Fab scaffold possesses one native methionine residue on its light chain and two native methionine residues on its heavy chain, none of these side chains are surface accessible and thus were not labeled by ReACT, even with high oxaziridine probe loadings (Fig. 3A and fig. S8). As such, because these native methionines are buried within the hydrophobic interior core, ReACT offers a potentially valuable strategy for Fab bioconjugation because there is no background labeling of the wild-type Fab and subsequent engineering of surface-accessible methionine sites can enable precise antibody functionalization at directed locations. Using the THIOMab platform (34), we demonstrated this possibility by replacing heavy chain (HC)-A114 or light chain (LC)-V205 residues with methionine and showed efficient labeling with ReACT (fig. S8). In addition, we established rapid, near-quantitative, and site-specific C-terminal labeling with ReACT on a GFP-Fab bearing a C-terminal methionine (GFP-Fab-CM) (Fig. 3, A and B). The resulting azide-carrying GFP-Fab (GFP-Fab-N<sub>3</sub>) retained similar binding affinity to the GFP ligand and compared to the wild-type Fab (fig. S9). Click reactions enabled further functionalization of GFP-Fab-N<sub>3</sub> with biotin, fluorophore, and drug payloads (Fig. 3B). Moreover, the resulting conjugates were compatible with biological environments. For example, we used a human embryonic kidney 293T (HEK-293T) cell line with a doxycycline (Dox)-inducible cell surface GFP expression system, where Dox treatment results in expression of GFP localized to the cell surface. Upon pre-addition of Dox followed by incubation with Cy3-labeled GFP-Fab made by ReACT, we observed excellent colocalization of Cy3 and GFP signals in live HEK-293T cells. In contrast, no Cy3 signal was observed in control cells without Dox addition (Fig. 3C). Furthermore, the intensity of the Cy3 signal was stable for at least 14 days in the presence of 100% fetal bovine serum (fig. S10). Taken together, these data demonstrate that ReACT can enable antibody functionalization at directed positions with a wide variety of payloads and simultaneously retain their function for ligand binding.

We next moved on to apply ReACT to a therapeutic conjugate, Herceptin-Fab (Her-Fab). As expected, ReACT did not label wild-type Her-Fab, owing to its lack of surface-accessible methionines (fig. S11). By engineering Her-Fab platforms carrying one or two methionine residues at the C terminus of the light chain, ReACT afforded near quantitative conjugation with one or two redox modifications, respectively (Fig. 3D). The data establish that ReACT can enable synthesis of ADCs with a defined drug-to-antibody ratio (DAR) in excellent purity, which remains a major challenge for bioconjugation methods employing cysteine or lysine ligation. Moreover, the bioorthogonal azide or alkyne handle introduced by methionine conjugation can be readily functionalized with additional payloads. The ADC synthesized by linking monomethyl auristatin E (MMAE)

to Her-Fab exhibited a fivefold increase in toxicity to Her2-positive BT474 breast cancer cells compared with either wild-type Her-Fab or a mixture of wild-type Her-Fab and free MMAE (Fig. 3E), demonstrating its utility in a biological context.

### Application of ReACT to chemoproteomic identification of functional methionines in cells

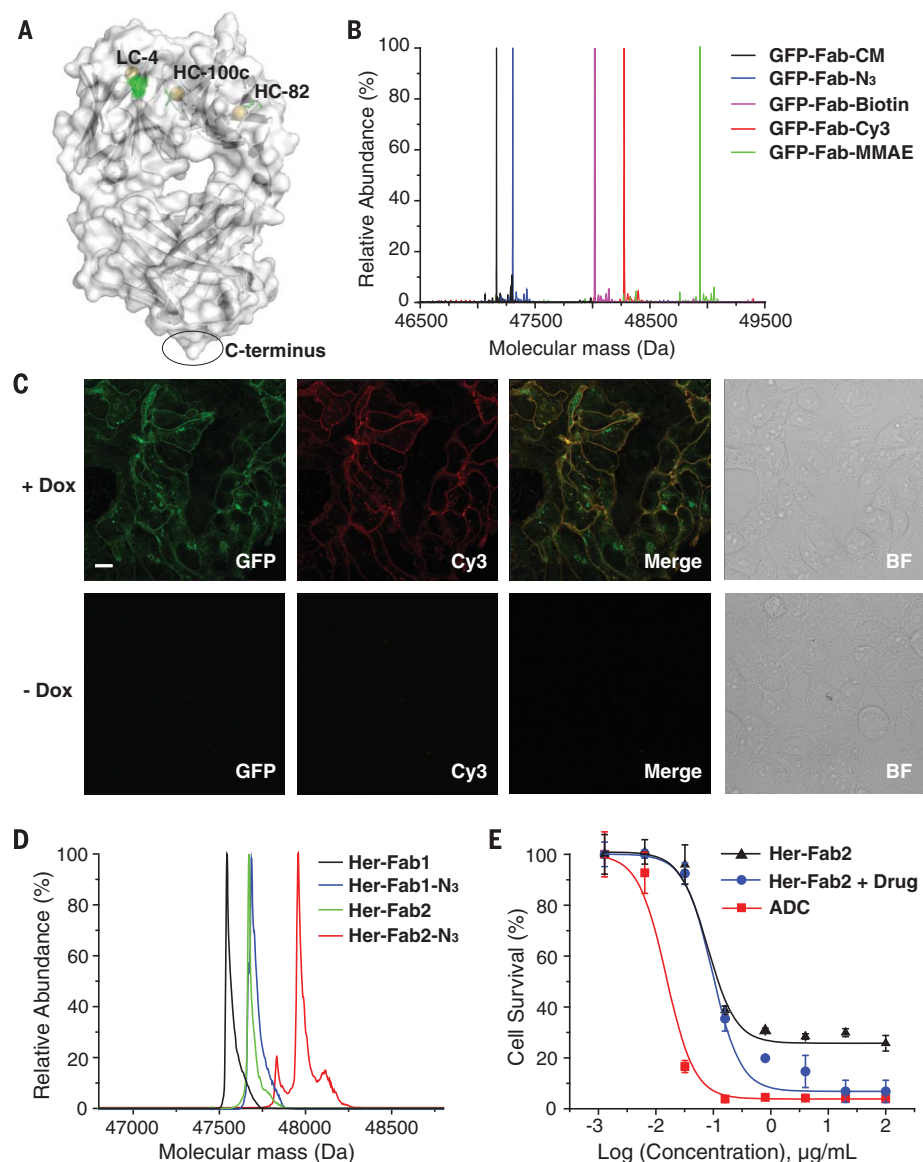
Finally, we turned our attention to the use of ReACT as a methionine-targeted warhead for chemoproteomics applications, owing to its high specificity and reactivity, as well as the small warhead size of the oxaziridine group (e.g., the molecular weight of Ox4 is 202 Da) that allows access to a broad range of proteins. To this end, we applied ReACT to probe reactive methionines in the proteome through tandem orthogonal proteolysis–activity–based protein profiling (TOP-ABPP) (35). Through dose-dependent treatment

of cells with low, medium, and high levels of ReACT probe Ox4 (Fig. 4A and fig. S12), we sought to identify hyperreactive methionines that should be enriched with low-dose labeling along with less-reactive methionine sites. By performing parallel TOP-ABPP ( $n = 2$  for all three groups) in HeLa cell lysates, we were able to identify 116 (low dose), 458 (medium dose), and 1111 (high dose) peptides that carry the desired ReACT methionine modification (Fig. 4, A and B, and Data S2). Compilation of the hyperreactive methionine-containing target proteins identified in the low-dose ReACT-treated group spanned many protein classes, including enzymes, chaperones, and nucleoproteins, as well as many structural proteins (Data S2). In general, only surface-accessible methionine residues were identified even with the high-dose probe, indicating that ReACT does not disrupt or denature proteins under these labeling conditions (Fig. 4C and fig. S13).

Of particular interest are the hyperreactive methionine targets, because they can predict sites of methionine-regulated protein function. This unbiased ReACT approach not only enables characterization of previously studied redox-sensitive methionines in whole-proteome settings but more importantly identifies new functional methionine sites. As a positive control, we identified three hyperreactive methionines within actin, including Met44 and Met47, whose redox activities have been previously shown to play a central role in controlling actin polymerization in living cells (Fig. 4C) (24, 25). With these data validating the ReACT method in hand, we moved on to identify and characterize new targets with methionine-dependent function. As one representative example, we found three hyperreactive methionine residues on enolase, a central enzyme in the ancient and conserved metabolic pathway of glycolysis (36), which is important in regulating

### Fig. 3. ReACT for synthesis of methionine-targeted antibody conjugates.

(A) Crystal structure of Her-Fab [Protein Data Bank (PDB) 1n8z] with three native methionine residues shown as green sticks and sulfur atoms shown as yellow spheres. The sulfur atoms appear to be buried in the pocket of Fab on the crystal structure. (B) The ratio of probe/methionine for Fab labeling is 10, with 10-min labeling time at room temperature. The deconvoluted MS data of GFP-Fab constructs with or without ReACT labeling. GFP-Fab-CM: expected mass 47158 Da, found 47158 Da; GFP-Fab-N<sub>3</sub> (labeled by Ox6): expected mass 47299 Da, found 47299 Da; biotin-functionalized GFP-Fab-N<sub>3</sub> (GFP-Fab-Biotin): expected mass 48019 Da, found 48019 Da; Cy3-functionalized GFP-Fab-N<sub>3</sub> (GFP-Fab-Cy3): expected mass 48282 Da, found 48282 Da. MMAE-functionalized GFP-Fab-N<sub>3</sub> (GFP-Fab-MMAE): expected mass 48949 Da, found 48949 Da. (C) Fluorescence colocalization imaging of GFP-Fab-Cy3 with cell surface-targeted GFP in HEK-293T cells. The GFP was inducibly expressed on the cell surface with addition of 1  $\mu$ M/mL of Dox before adding GFP-Fab-Cy3. Cells without addition of Dox were used as a control and show no antibody staining. All images use the same scale bar: 20  $\mu$ m. (D) The deconvoluted MS data of Her-Fab constructs with or without ReACT labeling. Her-Fab carries one C-terminal methionine (Her-Fab1): expected mass 47544 Da, found 47544 Da; Ox6-labeled Her-Fab1 (Her-Fab1-N<sub>3</sub>): expected mass 47685 Da, found 47686 Da; Her-Fab carries two C-terminal methionine (Her-Fab2): expected mass 47676 Da, found 47677 Da; Ox6-labeled Her-Fab2 (Her-Fab2-N<sub>3</sub>): expected mass 48958 Da, found 47958 Da. (E) In vitro cytotoxicity of Her-Fab2 [median effective concentration (EC<sub>50</sub>) = 0.086  $\pm$  0.02  $\mu$ g/mL], noncovalent mixture of Her-Fab2 and free MMAE (EC<sub>50</sub> = 0.096  $\pm$  0.04  $\mu$ g/mL), and the ReACT-derived ADC from Her-Fab2 and MMAE (EC<sub>50</sub> = 0.015  $\pm$  0.007  $\mu$ g/mL). Error bars, mean  $\pm$  SD from three independent experiments. EC<sub>50</sub> values and EC<sub>50</sub>  $\pm$  SDs were determined using four-parameter logistic fitting.

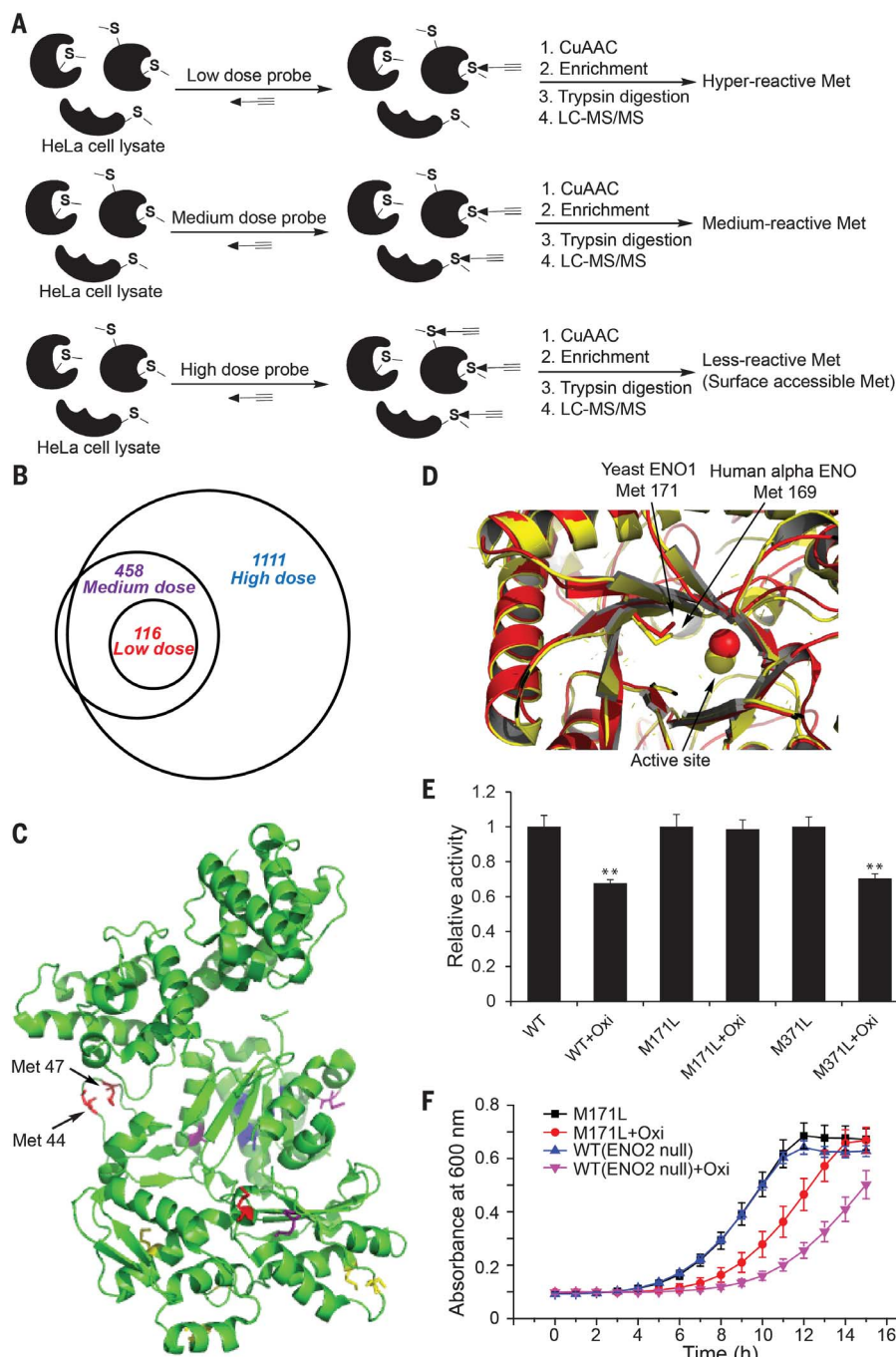


diseases such as cancer via the Warburg effect (37). In this scenario, cancer cells predominantly produce more energy compared with nontumorigenic cells by manipulating glycolysis enzymes (e.g., enolases). Among these three methionine residues in enolase, Met169 residue is highly conserved from yeast to mammals (corresponding to Met171 on yeast enolase 1) (Fig. 4D and fig. S14). Moreover, this residue is close to the enzyme active site and can be oxidized along with other methionine residues in the mammalian protein upon oxidant treatment (Fig. 4D and fig. S15).

To characterize the functional importance of this oxidation-sensitive methionine in enolase in

more detail, we cloned and purified a yeast homolog for *in vitro* biochemistry studies. Treatment of the wild-type yeast enolase 1 with hypochlorite decreased enzymatic activity with concomitant oxidation of methionine residues, including Met171, on the protein (Fig. 4E). A similar decrease in protein activity was observed upon oxidation of the M371L mutant. In contrast, the activity of the M171L mutant was unaffected by oxidant treatment under the same conditions (Fig. 4E), suggesting that this highly conserved residue is critical for redox regulation of enolase function. Kinetics measurements of the wild type and M171L mutants with and without oxidant treatment

revealed that both the turnover number ( $K_{cat}$ ) and the Michaelis constant ( $K_m$ ) are affected in the wild type upon oxidation but that these values for the M171L mutant remain the same (fig. S15). To show the physiological consequences of this methionine-based redox regulation at the cellular level, we generated yeast strains with a double enolase 1 and enolase 2 knockout background (38) and reintroduced either wild-type enolase 1 or the M171L mutant. As depicted in Fig. 4F, we observed that the strain carrying the M171L mutation is more resistant to oxidative stress-induced cell death compared to the strain with wild-type enolase 1, establishing that



**Fig. 4. Chemoproteomic methionine profiling with ReACT.**

(A) Reactive methionine profiling with ReACT involves treatment of proteomes with low, medium, and high doses of Ox4 probe, followed by CuAAC-based installation of acid cleavable biotin-azide tag, enrichment with streptavidin magnetic beads, and sequential on-bead trypsin digestions to afford probe-labeled peptides for LC-MS/MS analysis. (B) The number of peptides carrying the desired ReACT modification on methionine and appearing in two independent runs from the low-, medium-, and high-dose groups is shown. (C) Reactive methionine map on actin (PDB 3byh), with hyperreactive methionines colored in red, including Met44 and Met47, medium-reactive methionines colored in purple; and less-reactive methionines colored in blue. The methionines colored in yellow represent residues identified by LC-MS/MS that do not carry redox modification. The domains carrying these yellow-colored methionines are involved in actin polymerization. Because of this activity, no desired modification is detected on these residues even when these are surface accessible on the protein x-ray crystal structure. (D) Protein structure alignment of human alpha enolase (yellow; PDB 2psn) and yeast enolase 1 (red; PDB 2AL1), with conserved methionine residues shown in stick representation. (E) The relative activity of yeast enolase 1 variants with or without treatment of NaClO (100  $\mu$ M). Error bars, mean  $\pm$  SD from four independent experiments. *P* values indicated in the figure represent results of an unpaired *t* test. (F) Growth curve of wild-type (WT) (ENO2 null) and ENO1-M171L (ENO2 null) strains, with or without treatment of NaClO (100  $\mu$ M). Knockout and mutation strains were generated by clustered regularly interspaced short palindromic repeats (CRISPR)–Cas9–mediated genome editing. Error bars, mean  $\pm$  SD from three independent experiments.

this methionine residue serves a functional redox-active role in vivo.

## Conclusion

To close, ReACT provides a unique and general redox-based approach to chemoselective methionine conjugation that complements the wealth of acid-base conjugation methods for modification of more nucleophilic amino acids such as cysteine, lysine, and serine. The relative rarity of methionine in surface-accessible forms, such as in the complementarity-determining region of antibodies (39) that show highly similar sequence homology, presages the potential utility of this method for functionalization of antibodies and their fragments as well as other proteins using naturally occurring amino acids that are native or readily introduced by standard site-directed mutagenesis. More broadly, the ReACT method enables installation of various payloads onto therapeutic and imaging proteins at well-defined positions and with excellent DAR. Finally, the selectivity of the oxaziridine warhead offers a chemical platform for identifying and studying functional methionines in whole proteomes, as well as a starting point for therapeutic interventions based on reactive methionine activation and/or inhibition.

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## SUPPLEMENTARY MATERIALS

www.sciencemag.org/content/355/6325/597/suppl/DC1  
Materials and Methods  
Figs. S1 to S26  
Data S1 and S2  
References (40–68)

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## Redox-based reagents for chemoselective methionine bioconjugation

Shixian Lin, Xiaoyu Yang, Shang Jia, Amy M. Weeks, Michael Hornsby, Peter S. Lee, Rita V. Nichiporuk, Anthony T. Iavarone, James A. Wells, F. Dean Toste and Christopher J. Chang (February 9, 2017)

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Editor's Summary

### Targeting proteins at the other sulfur

As the only amino acid with a thiol (SH) group, cysteine is easily targeted for site-selective protein modifications. Hydrophobic methionine also has sulfur in its side chain, but its capping methyl group has hindered analogous targeting efforts. Lin *et al.* introduce a complementary protocol to tether new substituents exclusively to methionine, even in the presence of cysteine. They used an oxaziridine group as an oxidant to form sulfimide (S=N) linkages. The approach allowed antibody-drug conjugation and chemoproteomic screening for reactive methionine surface residues.

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