Bioorthogonal Chemistry

DOI: 10.1002/anie.201205352

Genetically Encoded Cyclopropene Directs Rapid, Photoclick-Chemistry-Mediated Protein Labeling in Mammalian Cells**

Zhipeng Yu, Yanchao Pan, Zhiyong Wang, Jiangyun Wang, and Qing Lin**

Dedicated to Professor Andrew D. Hamilton on the occasion of his 60th birthday

The introduction of bioorthogonal organic reporters into proteins site-selectively through genetic,[1] metabolic,[2] or enzyme-catalyzed ligation methods,[3] in conjunction with a growing repertoire of bioorthogonal reactions,[4] has allowed for the visualization and regulation of proteins in their native environment.[5] Numerous small organic functional groups, such as ketones,[6] azides,[7] terminal alkynes,[8] and terminal alkenes,[9] as well as larger reactive bioorthogonal groups, such as cyclooctyne,[10] norbornene,[11] transcyclooctene,[10b,11b] tetrazole,[12] and tetrazine[13] have been genetically encoded for site-selective protein labeling in vivo. To track fast protein dynamics in vivo, it is imperative that these genetically encoded bioorthogonal reporters direct fast and selective bioorthogonal labeling with the cognate biophysical probes, preferably with spatiotemporal control.

In our continued effort to genetically encode substrates that are suitable for photoclick chemistry,[14] we envisioned that non-natural amino acids with strained alkenes attached may show higher rate of cycloaddition without undergoing the Michael addition side reactions with biological nucleophiles typically associated with electron-deficient alkenes. While we reported recently that norbornene exhibited robust reactivity in the cycloaddition reaction with the macrocyclic tetrazoles,[15] norbornene is relatively bulky and may perturb the structure of the encoded protein. Therefore, we tried to genetically encode cyclopropene because of its small size and inherently high ring strain (54.1 kcal mol^{-1} [16] versus 21.6 kcal mol^{-1} for norbornene[17]), much of which is released after the cycloaddition reaction (ring strain of cyclopropane = 28.7 kcal mol^{-1}).[18] Herein, we report the synthesis of a stable cyclopropene amino acid, the characterization of its reactivity in the photoinduced cycloaddition reaction with two tetrazoles, its site-specific incorporation into proteins both in *E. coli* and in mammalian cells, and its use in directing bioorthogonal labeling of proteins both in vitro and in vivo.

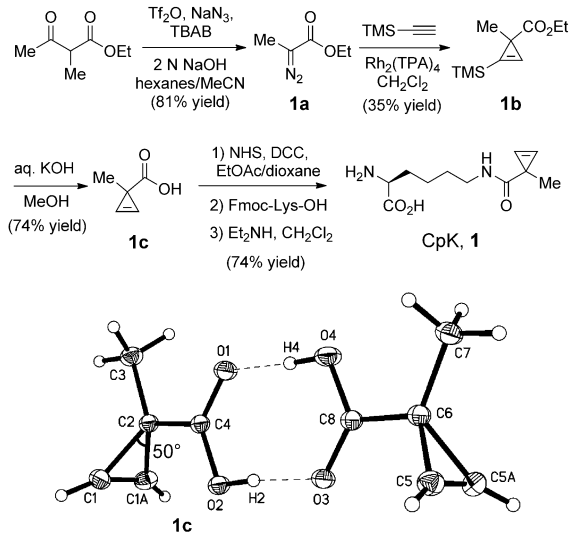
To design a cyclopropene-containing amino acid suitable for genetic incorporation, we decided to focus on pyrrolysyl/tRNA synthetase (PylRS)/tRNA$_{CUA}$ pair from *Methanosarcina barkeri* (Mb) because: 1) this pair is orthogonal to all endogenous tRNAs and aminoacyl-tRNA synthetases in *E. coli* and eukaryotic cells;[19] and 2) many non-natural lysine-derived amino acids have been efficiently incorporated into proteins based on this pair.[20] Preliminary studies showed that 3,3-disubstituted cyclopropenes such as **1c** exhibited excellent chemical stability at room temperature. 1-Methylcycloprop-2-enecarboxylic acid (**1c**) can be easily prepared from the commercially available starting material ethyl-2-methylacetoacetate, through a three-step procedure with an overall yield of 21% (Scheme 1). The cyclopropene carboxylic acid **1c** was then coupled with the ε-amino group of Fmoc-lysine, which upon removal of the protecting group afforded N^{ε}-(1-methylcycloprop-2-enecarboxamido)lysine (CpK, **1**) in 74% yield over three steps. The crystal structure of **1c** was obtained, which showed a hydrogen-bonded dimer of **1c** (Scheme 1; see Supporting Information, Table S1 for crystal data and structure refinement). As expected, a bond angle of only 50° at C1−C2−C1A provides very high strain in the ring structure. Importantly, the carbonyl group in **1c** is essentially perpendicular to cyclopropene double bond, preventing conjugation between these two π systems. Perhaps as a result of this geometry, CpK was found to be very stable around glutathione, an abundant biological nucleophile inside cells; greater than 95% of CpK remained after incubation with 10 mM glutathione (in reduced form) for more than 60 h (Supporting Information, Figure S1).

Using ethyl-1-methylcycloprop-2-enecarboxylate (**2**) as a model substrate, we examined the reactivity of cyclopropene with two representative tetrazoles: a 302 nm wavelength photoreactive tetrazole **3**[21] and a water-soluble 365 nm wavelength photoreactive tetrazole **4**,[22] in acetoni-

[*] Y. Pan,[+] Prof. Dr. J. Wang
Laboratory of Noncoding RNA, Institute of Biophysics, Chinese Academy of Sciences
Beijing 100101 (P.R. China)
E-mail: jwang@ibp.ac.cn
Dr. Z. Yu,[+] Dr. Z. Wang, Prof. Dr. Q. Lin
Department of Chemistry, State University of New York at Buffalo
Buffalo, NY 14260 (USA)
E-mail: qinglin@buffalo.edu
[+] These two authors contributed equally to the work.

[**] We gratefully acknowledge the National Institutes of Health (GM 085092 to Q.L.), the Major State Basic Research Program of China (2010CB912301 and 2009CB825505 to J.W.), National Science Foundation of China (90913022 to J.W.), and CAS (KZCX2-YW-JC101 and KSCX2-EW-G-7 to J.W.) for financial support. We thank William Brennessel at the University of Rochester for X-ray crystallography and Reyna K. Lim in the Q.L. lab for plasmid preparation, Alan Siegel at SUNY Buffalo Biological Sciences Imaging Facility (supported by National Science Foundation Major Research Instrumentation grant DBI-0923133) for assistance in microscopy. CCDC 808108 (**1c**) contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

Supporting information for this article (experimental details) is available on the WWW under http://dx.doi.org/10.1002/anie.201205352.



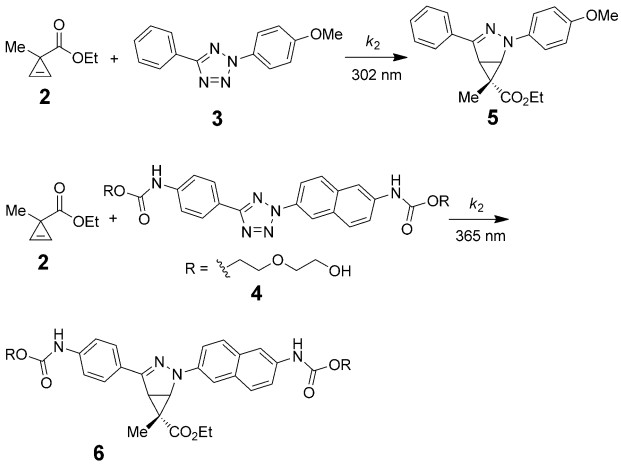
Scheme 1. Synthesis of N^{ε}-(1-methylcycloprop-2-enecarboxyamido)-lysine (CpK, **1**). Crystal structure of **1c** (below). Tf = triflate; TBAB = tetra-*n*-butyl ammonium bromide; TMS = trimethylsilyl; TPA = triphenylacetato; NHS = *N*-hydroxysuccinimide; DCC = dicyclohexylcarbodiimide; Fmoc = fluorenylmethyloxycarbonyl.

trile/PBS buffer (1:1) mixture. We compared the reactivity of this cyclopropene to those of acrylamide and norbornene (Table 1 and Figure S2–S7). In the cycloaddition reactions with tetrazole **3**, cyclopropene **2** showed a k_2 value of $58 \pm 16\,\text{M}^{-1}\text{s}^{-1}$, similar to acrylamide (entry 1 vs. 3) but significantly faster than norbornene (entry 1 vs. 5). In the cycloaddition reactions with tetrazole **4**, however, cyclopropene **2** showed more than a tenfold drop in k_2 value (entry 1 vs. 2), which is also 50% slower than that of acrylamide (entry 2 vs. 4) but similar to norbornene (entry 2 vs. 6). The decrease in reaction kinetics at the longer wavelength is likely due to the filtering effect associated with the formation of fluorescent pyrazoline adducts.[23] Compared to other bioorthogonal reactions involving genetically encoded alkenes, the cycloaddition of cyclopropene with tetrazole **3** is about 60 times faster than the cycloaddition of *O*-allyltyrosine under the same conditions ($k_2 = 0.95\,\text{M}^{-1}\text{s}^{-1}$),[21] at least six times faster than the ligation between 5-norbornene-2-ol and a pyrimidine-substituted tetrazine ($k_2 = 9\,\text{M}^{-1}\text{s}^{-1}$ in 95:5 H_2O/MeOH),[11a] but three orders of magnitude slower than tetrazine ligation with transcyclooctene ($k_2 = 35\,000 \pm 3000\,\text{M}^{-1}\text{s}^{1}$ in biological buffer).[11b]

To evolve an orthogonal tRNA/aminoacyl-tRNA synthetase pair that selectively charges CpK in response to a TAG amber codon in proteins in *E. coli*, an *Mb*PylRS library was constructed in which five residues (L266, L270, Y271, L274, and C313) were randomized through overlapping extension PCR using synthetic oligonucleotide primers (Table S2). After three rounds of positive and two rounds of negative selections, a CpK-specific aminoacyl-tRNA synthetase, termed CpKRS, was identified. Sequencing of this clone revealed the following five mutations: L266M, L270I, Y271L, L274A, and C313I. To test whether the CpKRS/*Mb*tRNA_{CUA} pair allows for efficient and selective CpK incorporation in *E. coli*, the expression of sperm whale myoglobin carrying an amber codon at position four and a C-terminal His tag was carried out in *E. coli* cells transformed with the CpKRS/*Mb*tRNA_{CUA} pair and grown in LB medium supplemented with 1 mM CpK. The CpK-encoded mutant myoglobin proteins (Myo-CpK) were obtained at a concentration of $3.0\,\text{mg}\,\text{L}^{-1}$. Importantly, Myo-CpK was produced only when CpK was added (Figure 1a), indicating that CpK incorporation is highly specific. ESI-TOF mass spectrometry showed a mass of 18476.0 Da for Myo-CpK (Figure S8), matching the calculated mass of 18476.3 Da. Subsequent tryptic digestion and tandem mass spectrometric analysis confirmed the presence of CpK at position four of the myoglobin (Figure 1b).

To assess whether CpK can serve as a bioorthogonal reporter for protein labeling, we incubated Myo-CpK with tetrazole **3** in PBS buffer and subjected the mixture to 302 nm photoirradiation with a handheld UV lamp for a period of 1–12 minutes. In-gel fluorescence analysis revealed the time-dependent appearance of fluorescence at the Myo-CpK band, with the highest intensity reached after 10 min (Figure 2a), consistent with the formation of fluorescent pyrazoline

Table 1: Kinetic characterization of ethyl-1-methylcycloprop-2-enecarboxylate (**2**) reactivity using photoclick chemistry in an acetonitrile/PBS (1:1) mixture.[a]



Entry	Alkene	Tetrazole	Photoactivation wavelength	Product	k_2 [$\text{M}^{-1}\text{s}^{-1}$]
1		**3**	302 nm	**5**	58 ± 16
2		**4**	365 nm	**6**	4.6 ± 1.3
3		**3**	302 nm	**7**	46 ± 8.6
4		**4**	365 nm	**8**	9.2 ± 0.7
5		**3**	302 nm	**9**	32 ± 12
6		**4**	365 nm	**10**	5.8 ± 1.4

[a] Reactions were set up by incubating 100 μM of tetrazole and 500 μM of alkene in 0.5 mL PBS/MeCN (1:1). The mixtures were exposed to a handheld UV lamp (302 nm: UVM-57, 0.16 A; 365 nm: UVGL-25, 0.16 A) for a specified time under ambient conditions. The product mixtures were analyzed by reversed-phase HPLC and quantified by comparing the integrated peaks to that of a standard. The kinetic experiments were repeated three times to derive the average kinetic constants along with the standard deviations. See Supporting Information, Figures S2–S7 for details.

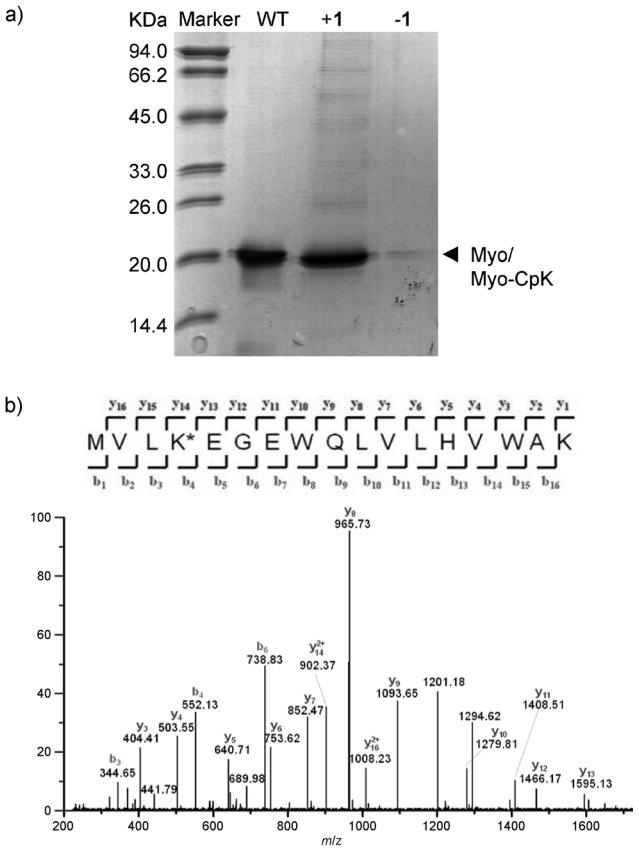


Figure 1. Incorporation of CpK (**1**) site-selectively into sperm whale myoglobin: a) Coomassie-blue-stained gel of the wildtype and mutant myoglobin expressed in the absence or presence of 1 mM CpK. A concentration of 3.0 mg L^{-1} was obtained for Myo-CpK. b) MS–MS spectrum of the N-terminal peptide fragment containing CpK (K*). The CpK mass of 207.48 Da was determined from the b_3 and b_4 ions (mass = $b_4 - b_3$), which closely matches the calculated mass of 208.13 Da.

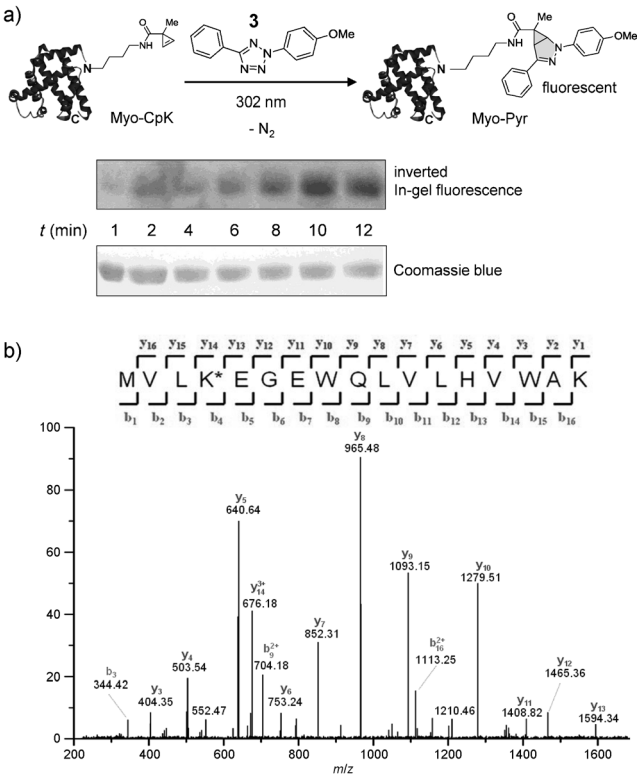


Figure 2. Selective labeling of CpK-encoded myoglobin using photoclick chemistry. a) Coomassie blue stain (bottom) and inverted fluorescence image (top) of the protein gel after the reaction mixtures were resolved by SDS-PAGE. For the photoclick reaction, a solution of Myo-CpK (0.5 mg mL^{-1}) and tetrazole **3** (100 μM) in PBS buffer was exposed to 302 nm wavelength photoirradiation for a period of 1–12 min. b) MS–MS spectrum of the N-terminal peptide fragment of the pyrazoline-modified myoglobin (Myo-Pyr) containing the pyrazoline-linked lysine (K*).

adduct (Figure 2a).[24] As a control, photoirradiation of a mixture of wild-type myoglobin and tetrazole **3** did not yield fluorescent bands on the SDS-PAGE gel (Figure S9), confirming that labeling of Myo-CpK was mediated by the cyclopropene moiety. Furthermore, ESI-TOF mass spectrometric analysis of the product mixture revealed greater than 85% conversion with a new mass peak at 18701.87 Da, matching the calculated mass of the pyrazoline-labeled myoglobin, 18701.56 (Figure S10). Tryptic digestion and tandem mass spectrometry confirmed the presence of the pyrazoline-modified lysine at position four by the appearance of expected masses of the three fragment ions b_9^{2+}, b_{16}^{2+}, and y_{14}^{3+} (Figure 2b). For comparison, the same photoclick reaction with a mutant myoglobin encoding the recently reported norbornene-modified lysine (NorK)[11a] at position four[25] showed a time-dependent, weakly fluorescent cycloadduct formation (Figure S12), affording the pyrazoline cycloadduct in 60% yield, based on mass spectrometric analysis, after ten minutes of photoirradiation (Figure S13). The slightly higher reactivity of CpK relative to NorK in the context of intact proteins is consistent with the kinetic data described in Table 1.

To examine whether CpK can direct bioorthogonal labeling in mammalian cells, we co-transfected human embryonic kidney (HEK) 293 cells with the pCMV-CpKRS plasmid, in which the transcription of CpKRS is under the control of a CMV promoter and the transcription of *Mb*tRNA$_{CUA}$ is under the control of a human U6 promoter,[20c] together with the pSwan-EGFP37TAG reporter.[20d] The cells were allowed to grow in the presence 4 mM CpK for 36 h, treated with 40 μM tetrazole **4** for 1.5 h followed by brief photoirradiation at 365 nm for two minutes, and finally imaged using a fluorescence microscope. Using the EGFP channel ($\lambda_{ex} = 488$ nm, $\lambda_{em} = 499$-578 nm), green fluorescent cells were detected only in plates where CpK was included in the culture medium (Figure 3b,e vs. Figure 3h), indicating that the CpKRS/*Mb*tRNA$_{CUA}$ pair supports site-specific incorporation of CpK into EGFP37TAG protein in HEK293 cells. Using the pyrazoline channel ($\lambda_{ex} = 405$ nm, $\lambda_{em} = 410$-498 nm), only the tetrazole **4**-treated cells expressing CpK-encoded EGFP showed cyan fluorescence (Figure 3a vs. Figure 3d,g; see Figure S14 for the fluorescence spectrum of the pyrazoline adduct). Notably, fluorescence images were acquired using two separate channels, with a single laser source exciting one wavelength a time to avoid any possible fluorescence leakage to the unintended channel. As seen in the overlaid images, the

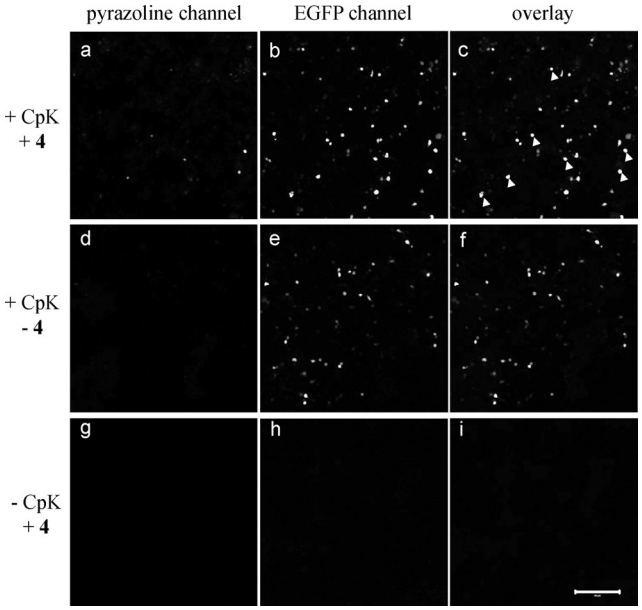


Figure 3. Confocal micrographs of human embryonic kidney 293 cells transfected with the plasmids expressing CpKRS, *Mb*tRNA$_{CUA}$, and EGFP37TAG, and grown in the presence of 4 mM CpK (a–f) or the absence of CpK (g–i). The cells in (a–c) and (h–j) were treated with 40 μM tetrazole **4** for 1.5 h before 365 nm wavelength photoirradiation with a handheld UV lamp for 2 min. White arrowheads indicate cells positive for both pyrazoline and EGFP fluorescence. Scale bar = 200 μm.

cyan fluorescent cells coincided with cells that showed high green fluorescence (indicated by white arrows in Figure 3c), suggesting that the labeling reaction was indeed directed by the cyclopropene moiety. However, not all green fluorescent cells were labelled, indicating some variability in the penetration of the tetrazole into the highly confluent HEK293 cells. Notably, repeated attempts to incorporate NorK in HEK293 cells using wild-type *Mb*PylRS and identical transfection conditions were not successful (Figure S15, S16), precluding the comparison of the reactivity of these two strained alkenes in photoclick chemistry in vivo.

In conclusion, we have demonstrated the genetic incorporation of a cyclopropene-containing amino acid, CpK, site-specifically into target proteins, and the use of CpK as a bioorthogonal reporter for directing rapid (approximately two minutes) fluorescent labeling of the target protein in mammalian cells. Compared to other genetically encoded, bioorthogonal labeling reactions reported recently,[10–13] the main advantage of the cyclopropene-directed photoclick chemistry lies in its potential for spatiotemporally controlled protein labeling in mammalian cells, which requires the development of highly reactive laser-activatable tetrazole reagents using either a single-photon (for example 405 nm) or two-photon laser source; work in this area is currently in progress. Because of its small size, a cyclopropene moiety such as **1c** can also be readily incorporated into small-molecule substrates and inhibitors for the study of proteomes[26] and lipids.[27]

Received: July 7, 2012
Revised: August 21, 2012
Published online: ■■ ■■, ■■■■

Keywords: bioorthogonal · click chemistry · cyclopropenes · proteins · tetrazoles

[1] C. C. Liu, P. G. Schultz, *Annu. Rev. Biochem.* **2010**, *79*, 413.
[2] a) E. Saxon, C. R. Bertozzi, *Science* **2000**, *287*, 2007; b) D. C. Dieterich, J. J. Hodas, G. Gouzer, I. Y. Shadrin, J. T. Ngo, A. Triller, D. A. Tirrell, E. M. Schuman, *Nat. Neurosci.* **2010**, *13*, 897.
[3] M. Fernández-Suárez, H. Baruah, L. Martínez-Hernández, K. T. Xie, J. M. Baskin, C. R. Bertozzi, A. Y. Ting, *Nat. Biotechnol.* **2007**, *25*, 1483.
[4] a) E. M. Sletten, C. R. Bertozzi, *Angew. Chem.* **2009**, *121*, 7108; *Angew. Chem. Int. Ed.* **2009**, *48*, 6974; b) R. K. Lim, Q. Lin, *Chem. Commun.* **2010**, *46*, 1589.
[5] E. M. Sletten, C. R. Bertozzi, *Acc. Chem. Res.* **2011**, *44*, 666.
[6] L. Wang, Z. Zhang, A. Brock, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 56.
[7] J. W. Chin, S. W. Santoro, A. B. Martin, D. S. King, L. Wang, P. G. Schultz, *J. Am. Chem. Soc.* **2002**, *124*, 9026.
[8] a) A. Deiters, T. A. Cropp, M. Mukherji, J. W. Chin, J. C. Anderson, P. G. Schultz, *J. Am. Chem. Soc.* **2003**, *125*, 11782; b) D. P. Nguyen, H. Lusic, H. Neumann, P. B. Kapadnis, A. Deiters, J. W. Chin, *J. Am. Chem. Soc.* **2009**, *131*, 8720; c) T. Fekner, X. Li, M. M. Lee, M. K. Chan, *Angew. Chem.* **2009**, *121*, 1661; *Angew. Chem. Int. Ed.* **2009**, *48*, 1633.
[9] a) Z. Zhang, L. Wang, A. Brock, P. G. Schultz, *Angew. Chem.* **2002**, *114*, 2964; *Angew. Chem. Int. Ed.* **2002**, *41*, 2840; b) W. Song, Y. Wang, J. Qu, Q. Lin, *J. Am. Chem. Soc.* **2008**, *130*, 9654; c) H. W. Ai, W. Shen, E. Brustad, P. G. Schultz, *Angew. Chem.* **2010**, *122*, 947; *Angew. Chem. Int. Ed.* **2010**, *49*, 935.
[10] a) T. Plass, S. Milles, C. Koehler, C. Schultz, E. A. Lemke, *Angew. Chem.* **2011**, *123*, 3964; *Angew. Chem. Int. Ed.* **2011**, *50*, 3878; b) K. Lang, L. Davis, S. Wallace, M. Mahesh, D. J. Cox, M. L. Blackman, J. M. Fox, J. W. Chin, *J. Am. Chem. Soc.* **2012**, *134*, 10317.
[11] a) K. Lang, L. Davis, J. Torres-Kolbus, C. Chou, A. Deiters, J. W. Chin, *Nat. Chem.* **2012**, *4*, 298; b) T. Plass, S. Milles, C. Koehler, J. Szymański, R. Mueller, M. Wießler, C. Schultz, E. A. Lemke, *Angew. Chem.* **2012**, *124*, 4242; *Angew. Chem. Int. Ed.* **2012**, *51*, 4166; c) E. Kaya, M. Vrabel, C. Deiml, S. Prill, V. S. Fluxa, T. Carell, *Angew. Chem.* **2012**, *124*, 4542; *Angew. Chem. Int. Ed.* **2012**, *51*, 4466.
[12] J. Wang, W. Zhang, W. Song, Y. Wang, Z. Yu, J. Li, M. Wu, L. Wang, J. Zang, Q. Lin, *J. Am. Chem. Soc.* **2010**, *132*, 14812.
[13] J. L. Seitchik, J. C. Peeler, M. T. Taylor, M. L. Blackman, T. W. Rhoads, R. B. Cooley, C. Refakis, J. M. Fox, R. A. Mehl, *J. Am. Chem. Soc.* **2012**, *134*, 2898.
[14] a) Y. Wang, Q. Lin, *Org. Lett.* **2009**, *11*, 3570; b) R. K. Lim, Q. Lin, *Acc. Chem. Res.* **2011**, *44*, 828.
[15] Z. Yu, R. K. Lim, Q. Lin, *Chem. Eur. J.* **2010**, *16*, 13325.
[16] R. D. Bach, O. Dmitrenko, *J. Am. Chem. Soc.* **2004**, *126*, 4444.
[17] P. v. R. Schleyer, J. E. William, K. R. Blanchard, *J. Am. Chem. Soc.* **1970**, *92*, 2377.
[18] M. S. Gordon, *J. Am. Chem. Soc.* **1980**, *102*, 7419.
[19] a) S. K. Blight, R. C. Larue, A. Mahapatra, D. G. Longstaff, E. Chang, G. Zhao, P. T. Kang, K. B. Green-Church, M. K. Chan, J. A. Krzycki, *Nature* **2004**, *431*, 333; b) A. Ambrogelly, S. Gundllapalli, S. Herring, C. Polycarpo, C. Frauer, D. Söll, *Proc. Natl. Acad. Sci. USA* **2007**, *104*, 3141.
[20] a) H. Neumann, S. Y. Peak-Chew, J. W. Chin, *Nat. Chem. Biol.* **2008**, *4*, 232; b) T. Mukai, T. Kobayashi, N. Hino, T. Yanagisawa, K. Sakamoto, S. Yokoyama, *Biochem. Biophys. Res. Commun.*

2008, *371*, 818; c) X. Li, T. Fekner, J. J. Ottesen, M. K. Chan, *Angew. Chem.* **2009**, *121*, 9348; *Angew. Chem. Int. Ed.* **2009**, *48*, 9184; d) P. R. Chen, D. Groff, J. Guo, W. Ou, S. Cellitti, B. H. Geierstanger, P. G. Schultz, *Angew. Chem.* **2009**, *121*, 4112; *Angew. Chem. Int. Ed.* **2009**, *48*, 4052; e) J. K. Takimoto, N. Dellas, J. P. Noel, L. Wang, *ACS Chem. Biol.* **2011**, *6*, 733; f) Y.-S. Wang, X. Fang, A. L. Wallace, B. Wu, W. R. Liu, *J. Am. Chem. Soc.* **2012**, *134*, 2950.

[21] Y. Wang, W. Song, W. J. Hu, Q. Lin, *Angew. Chem.* **2009**, *121*, 5434; *Angew. Chem. Int. Ed.* **2009**, *48*, 5330.

[22] Z. Yu, L. Y. Ho, Z. Wang, Q. Lin, *Bioorg. Med. Chem. Lett.* **2011**, *21*, 5033.

[23] Y. Wang, W. J. Hu, W. Song, R. K. Lim, Q. Lin, *Org. Lett.* **2008**, *10*, 3725.

[24] W. Song, Y. Wang, J. Qu, M. M. Madden, Q. Lin, *Angew. Chem.* **2008**, *120*, 2874; *Angew. Chem. Int. Ed.* **2008**, *47*, 2832.

[25] The NorK-encoded myoglobin mutant was expressed using wildtype pylRS and a pBAD-pylT-Myo4TAG reporter in *E. coli* in the presence of 1 mM NorK. A yield of the isolated product of 1.0 mg L^{-1} was obtained for the NorK-containing myoglobin (see Figure S11 for characterization data).

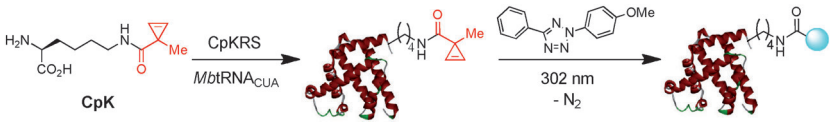
[26] B. R. Martin, C. Wang, A. Adibekian, S. E. Tully, B. F. Cravatt, *Nat. Methods* **2011**, *9*, 84.

[27] During the preparation of this manuscript, a report on the use of 1-methylcyclopropene as a lipid reporter appeared: J. Yang, J. Sečkutė, C. M. Cole, N. K. Devaraj, *Angew. Chem.* **2012**, *124*, 7594; *Angew. Chem. Int. Ed.* **2012**, *51*, 7476.

Communications

Bioorthogonal Chemistry

Z. Yu, Y. Pan, Z. Wang, J. Wang,*
Q. Lin* _____ ■■■■–■■■■



Genetically Encoded Cyclopropene Directs Rapid, Photoclick-Chemistry-Mediated Protein Labeling in Mammalian Cells

We just click: Genetic incorporation of a cyclopropene amino acid CpK (see scheme) site-specifically into proteins in *E. coli* and mammalian cells was achieved using an orthogonal aminoacyl-tRNA synthetase/$tRNA_{CUA}$ pair (CpKRS/$MbtRNA_{CUA}$). Cyclopropene exhibited fast reaction kinetics in the photoclick reaction and allowed rapid (ca. 2 min) labeling of proteins.