Protein Engineering

DOI: 10.1002/anie.201400001

Genetically Encoding Photoswitchable Click Amino Acids in *Escherichia coli* and Mammalian Cells**

*Christian Hoppmann, Vanessa K. Lacey, Gordon V. Louie, Jing Wei, Joseph P. Noel, and Lei Wang**

Abstract: *The ability to reversibly control protein structure and function with light would offer high spatiotemporal resolution for investigating biological processes. To confer photoresponsiveness on general proteins, we genetically incorporated a set of photoswitchable click amino acids (PSCaas), which contain both a reversible photoswitch and an additional click functional group for further modifications. Orthogonal tRNA-synthetases were evolved to genetically encode PSCaas bearing azobenzene with an alkene, keto, or benzyl chloride group in E. coli and in mammalian cells. After incorporation into calmodulin, the benzyl chloride PSCaa spontaneously generated a covalent protein bridge by reacting with a nearby cysteine residue through proximity-enabled bioreactivity. The resultant azobenzene bridge isomerized in response to light, thereby changing the conformation of calmodulin. These genetically encodable PSCaas will prove valuable for engineering photoswitchable bridges into proteins for reversible optogenetic regulation.*

Photoswitchable biomolecules, most commonly peptides and proteins, are finding increasing utility in the in vivo investigation of biological processes, where they confer a minimally invasive means for precise spatiotemporal control.[1] Of particular interest are photoisomerizable functionalities, which can induce reversible changes in protein conformation and bioactivity.[2] Azobenzene photoswitches are very effective for this application because photoinduced *trans*↔*cis* isomerization around the central N=N bond occurs with high yield and can drive remarkable structural changes in vitro and even in vivo.[3,4] Until now, optical control of protein conformation required the use of a bifunctional, thiol-reactive azobenzene reagent, which can crosslink two suitably situated cysteine resides within a protein to generate a photoswitchable bridge of the side chains.[5,6] However, the utility of this approach in vivo has been limited by the high reactivity of the crosslinking reagents and the lack of specificity in targeting the modification, factors that lead to the abundant formation of undesired products. To overcome these limitations, we recently developed a photoswitchable click amino acid (PSCaa **1**; Figure 1 A) that allows the site-specific introduction of a photoswitchable bridge. Through thiol-click chemistry, the bridge can be formed under mild conditions and is selectively formed between the alkene of **1** and the thiol group of a nearby cysteine, even in the presence of other thiols.[7] Previously, we chemically synthesized peptides that incorporate a PSCaa-mediated photocontrollable bridge and demonstrated that the helical conformation of the peptides could be optically regulated, thereby leading to differing bioactivities.[7] The site-specific

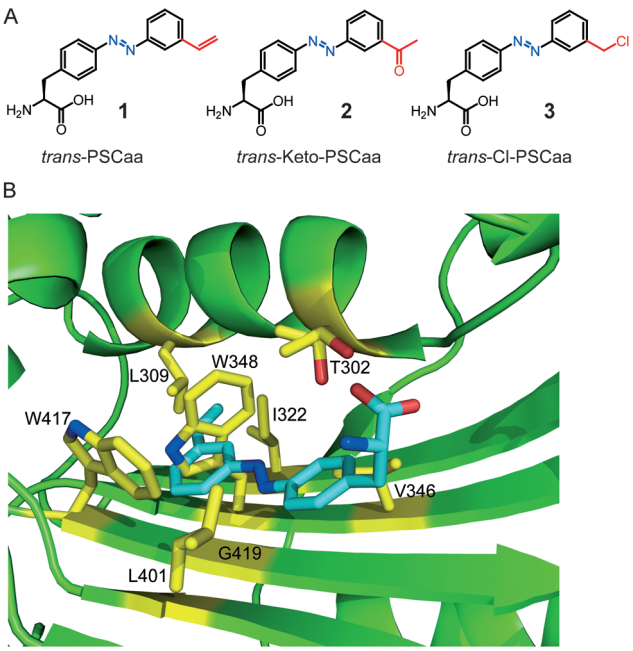

Figure 1. A) The *trans* forms of the photoswitchable click amino acids. PSCaa **1**: 2-amino-3-(4-((3-vinylphenyl)diazenyl)phenyl)propanoic acid; Keto-PSCaa **2**: 2-amino-3-(4-((3-(2-acetyl)phenyl)diazenyl)phenyl)propanoic acid; Cl-PSCaa **3**: 2-amino-3-(4-((3-(chloromethyl)phenyl)diazenyl)phenyl)propanoic acid. B) X-ray crystal structure of the active site cavity of MmOmePylRS (PDB 3QTC), in which the structure of PSCaa **1** (cyan) was superimposed. Residues mutated in the synthetase library are shown as yellow stick models.

[*] Dr. C. Hoppmann, Dr. V. K. Lacey, Dr. G. V. Louie, Prof. J. P. Noel, Prof. L. Wang
Chemical Biology and Proteomics Laboratory
The Salk Institute for Biological Studies
10010 N. Torrey Pines Road, La Jolla, CA 92037 (USA)
E-mail: lwang@salk.edu
Homepage: http://wang.salk.edu
J. Wei
Jadebio, Inc.
La Jolla, CA 92037 (USA)
Prof. J. P. Noel
Howard Hughes Medical Institute (USA)

[**] C.H. gratefully acknowledges a fellowship from the Deutsche Forschungsgemeinschaft (DFG; HO 4778/1-1) and partial funding from the Pioneer Fund. L.W. acknowledges support from California Institute for Regenerative Medicine (RN1-00577-1) and US National Institutes of Health (1DP2OD004744-01 and P30CA014195).
Supporting information for this article is available on the WWW under http://dx.doi.org/10.1002/anie.201400001.

incorporation of PSCaas into recombinant proteins in situ will enable the study of protein function and biological processes through light modulation in living cells.[8] Although phenylazophenylalanine (AzoPhe) has been incorporated into proteins in *E. coli*,[9] the tRNA–synthetase pair evolved for AzoPhe cannot be used in mammalian cells. Moreover, the AzoPhe side chain lacks a reactive functional group for further derivatization and thus cannot mediate bridge formation. Therefore, light-induced isomerization of AzoPhe may induce only localized structural perturbations and not large-scale changes in protein conformation. To date, there have been no reports of the use of azobenzene units for the photocontrol of protein structural states in living cells.

Herein, we describe the generation of orthogonal tRNA–synthetase pairs to incorporate azobenzene-based photoswitchable click amino acids into proteins in both *E. coli* and mammalian cells in a site-specific manner. Using a semirational approach for the construction of a mutant library of the synthetase (*Methanosarcina mazei* PylRS, MmPylRS), we were able to simultaneously mutate a greater number of residues in comparison to a more conventional approach that employs full randomization at each site. The increased diversity of the MmPylRS library enabled us to evolve mutant synthetases specific for AzoPhe derivatives bearing an additional alkene (PSCaa **1**), keto (Keto-PSCaa **2**), or benzyl chloride (Cl-PSCaa **3**) group (Figure 1 A). In addition, we generated a protein bridge by utilizing the proximity-enabled bioreactivity[10] of Cl-PSCaa **3** toward cysteine and showed photoswitching of the resultant azobenzene-containing bridge to alter protein conformation. This work demonstrates the first genetic incorporation into proteins of an unnatural amino acid (Uaa) that contains both the azobenzene photoswitch and an additional click functionality, and forms the foundation for the generation of a designed recombinant protein carrying a photocontrollable bridge in living cells.

To evolve a mutant synthetase specific for **1**, we initially generated five different synthetase libraries by fully randomizing five or six residues in the active site of MmPylRS through conventional NNK codon randomization.[11] Although these libraries yielded mutants specific for other Uaas,[12] no mutant could be identified for **1**, presumably because more substantial changes to the synthetase active site are required to accommodate the greater length and rigidity of **1**. Simultaneous mutation of a larger number of synthetase residues in *E. coli* necessitated that we use a semirational strategy, in which certain sites are restricted to a selected subset of the twenty amino acids. Using the structure of the MmOmeRS/*O*-methyl-L-tyrosine (Ome) complex as a guide,[12] we superimposed **1** onto Ome and decided to mutate residues 302, 309, 322, 346, 348, 401, 417, and 419 (Figure 1 B). Positions 309, 322, and 417 were fully randomized with the NNK codon. Based on its known importance in the recognition of Phe analogues,[12,15] residue 302 was allowed to be Thr, Ser, or Ala. Positions neighboring the azobenzene moiety were restricted to small, aliphatic residues: Val/Leu/Ala for residues 346 and 401, and Gly/Val/Ala for residues 348 and 419. The theoretical gene library size was 4×10^7 mutants, and an *E. coli* library of 6×10^8 colony-forming units was made.

One round of positive selection was performed in an *E. coli* strain (DH10β) carrying the selection plasmid pREP, which encodes both green fluorescent protein (GFP) and chloramphenicol acetyltransferase reporter cassettes containing the TAG stop codon.[13] Cells were grown on minimal medium containing 1 mM PSCaa **1** and 40 μg mL^{-1} chloramphenicol. A total of 60 green fluorescent colonies were obtained and subsequently screened by streaking on minimal-medium plates supplemented with chloramphenicol (60 μg mL^{-1}) in the presence or absence of 1 mM PSCaa. Thirteen clones showed PSCaa-dependent survival and green fluorescence (Figure S1A in the Supporting Information). To determine the efficiency of PSCaa incorporation by these clones, a mutant GFP gene containing a TAG stop codon at position 182 (GFP_182TAG) was expressed in *E. coli* in 2xYT medium by using the corresponding $tRNA^{Pyl}_{CUA}$–PSCaaRS pairs. PSCaa-dependent GFP expression was observed for 12 clones (Figure S1B).

DNA sequencing revealed that these clones represent seven distinct synthetase mutants (Table S1 in the Supporting Information). All seven mutants carried the same amino acid substitutions at positions 348, 401, 417, and 419, whereas some variability was seen at positions 302, 309, 322, and 346. Based on GFP expression in the presence or absence of PSCaa **1** (Figure S1B), we chose the clone that showed the highest selectivity for incorporating the PSCaa over natural amino acids. The mutant synthetase encoded by this clone, named MmPSCaaRS, carried the amino acid identities Thr302, Ser309, Ile322, Val346, Gly348, Tyr384, Val401, Trp417, and Gly419, and was characterized further.

To evaluate the efficiency and fidelity of the evolved MmPSCaaRS to incorporate PSCaa **1**, we expressed a gene for sperm whale myoglobin (Myo_4TAGHis6, encoding an amber TAG codon at position 4 and a C-terminal Hisx6 tag) together with the $tRNA^{Pyl}_{CUA}$–MmPSCaaRS in BL21 cells. From SDS-PAGE analysis of the expressed products, full-length myoglobin was obtained in good yield (ca. 1.8 mg mL^{-1}) in the presence of 1 mM PSCaa **1** but was undetectable in the absence of PSCaa **1** (Figure 2 A). The myoglobin purified from this expression experiment was analyzed by electrospray ionization Fourier transform ion trap mass spectrometry (ESI-FTMS). An observed peak with a monoisotopic mass of 18608.76 Da (Figure 2 B) corresponds to intact myoglobin with a single PSCaa residue at position 4 (expected $[M+H]^+ = 18608.81$ Da). A second observed peak corresponds to the PSCaa-containing myoglobin lacking the initiator Met (expected $[M-Met+H]^+ = 18477.77$ Da, measured 18477.72 Da). No peaks corresponding to myoglobin proteins containing any of the natural amino acids at the amber-codon position were observed. Furthermore, no hydrazo myoglobin resulting from the reduction of the azo bond was detected, thus indicating that the PSCaa incorporated into myoglobin remains stable under the reducing conditions of the *E. coli* cytosol.

To investigate whether PSCaa **1** could be incorporated into proteins in mammalian cells, we transfected genes for the $tRNA^{Pyl}_{CUA}$–MmPSCaaRS pair into a HeLa-GFP reporter cell line, in which the GFP_182TAG gene is stably integrated into the genome.[12,14] Green fluorescence of GFP appeared only

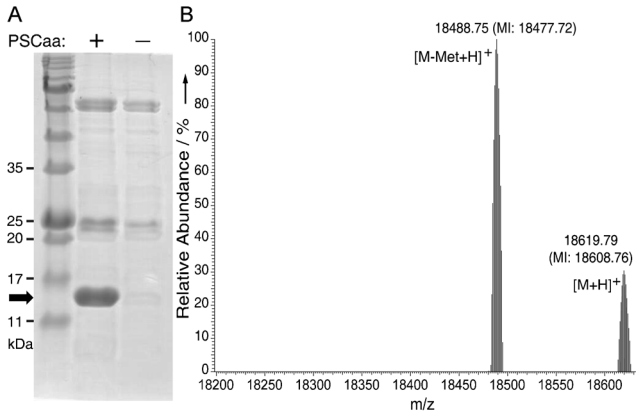

Figure 2. A) SDS-PAGE with Coomassie staining shows that $tRNA^{Pyl}_{CUA}$–MmPSCaaRS specifically incorporated PSCaa **1** into myoglobin in *E. coli* by suppression of the amber codon. Samples were normalized for constant cell numbers for each lane. The arrow indicates the position of full-length myoglobin. B) High resolution ESI-FTMS analysis of intact myoglobin expressed in the presence of $tRNA^{Pyl}_{CUA}$–MmPSCaaRS supplemented with 1 mM PSCaa **1** in nutrient rich growth media. Only the Uaa was found to be incorporated at the TAG encoded position. Average and monoisotopic (MI) masses are labeled.

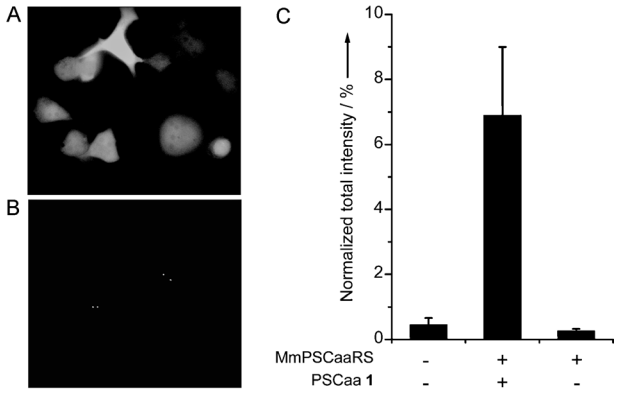

Figure 3. Incorporation of PSCaa **1** into GFP in mammalian cells by the $tRNA^{Pyl}_{CUA}$–MmPSCaaRS pair. A) Fluorescence imaging of the HeLa-GFP(182TAG) reporter cell line transfected with the $tRNA^{Pyl}_{CUA}$–MmPSCaaRS and grown in the presence of 0.1 mM PSCaa **1**. Green fluorescence was detected, thus indicating in vivo incorporation of the PSCaa into GFP. B) No fluorescence was detected in cells cultured in medium lacking PSCaa **1**. C) Incorporation efficiency of PSCaa **1** measured with flow cytometry. The total fluorescence intensity of cells was measured and normalized to cells transfected with the $tRNA^{Pyl}_{CUA}$–PylRS (wt) pair and supplemented with Boc-Lys. The same number of cells was analyzed for each sample. Error bars represent the standard error of the mean (SEM). The value (mean ± SEM) is 6.9 ± 2.5 % ($n = 3$) for PSCaa **1**.

when cells were grown in the presence of PSCaa **1** (Figure 3 A and B), thus indicating the selective incorporation of **1** into GFP. The relatively low concentration (0.1 mM) of **1** in the growth medium that was sufficient to support GFP expression suggests efficient cellular uptake of this hydrophobic Uaa into mammalian cells. GFP expression was quantified through measuring cellular fluorescence by using flow cytometry (Figure 3 C). The total fluorescence intensity of each sample was normalized to that of the HeLa-GFP reporter cells transfected with the wild type (wt) *M. maizei* $tRNA^{Pyl}_{CUA}$–PylRS pair and supplemented with N^{ε}-*tert*-butyloxycarbonyl-L-lysine (Boc-Lys), which is efficiently incorporated by the wt MmPylRS. The incorporation efficiency for PSCaa **1** by the $tRNA^{Pyl}_{CUA}$-MmPSCaaRS pair was approximately 7 % of wt, with a background suppression of only 0.2 % detected in the absence of PSCaa.

To understand how the PSCaa is recognized by the evolved synthetases, we structurally modeled MmPSCaaRS and the binding of PSCaa **1** by using the MmOmeRS/Ome complex structure as the template (Figure 4). In agreement with previously characterized PylRS mutants evolved to recognize other Phe analogues,[12,15] Thr302 in MmPSCaaRS (or the equivalent Ser302 in other PSCaa-incorporating mutants) forms hydrogen bonds with the α-carboxylate group of the PSCaa. The azobenzene moiety of the PSCaa occupies a hydrophobic pocket formed by G348, V401, W417, and G419, which are conserved in all of the identified mutants (Table S1). The alkene group of PSCaa is in contact with residues 309 and 322, for which diverse substitutions were identified in the PSCaa-incorporating mutants.

The tolerance of multiple substituting residues at the 309 and 322 sites suggests that the alkene group of PSCaa can be accommodated by several distinct combinations of amino acid residues and that its binding interactions contribute

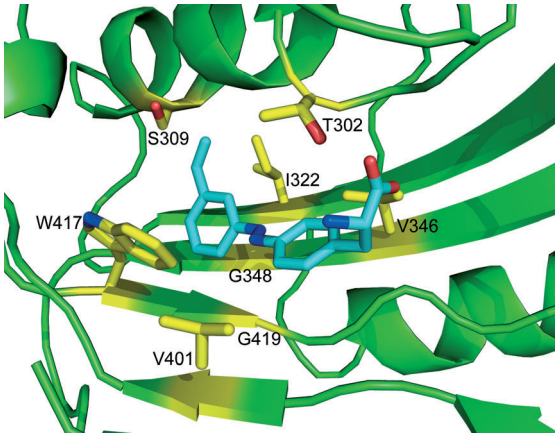

Figure 4. Molecular docking of PSCaa **1** into a model of MmPSCaaRS, which was built from 3QTC bearing the identified substitutions. Notably, positions 348 and 419 at the bottom of the substrate binding pocket were restricted to Gly to allow the long planar azobenzene moiety to extend into the active site. The mutation of residue 309 into A/S/G shortens the side chain to possibly accommodate the extended alkene group. T or S at position 302 form hydrogens bonds with the α-carboxyl group of the PSCaa.

secondarily to the recognition of the PSCaa. We therefore reasoned that azobenzene Uaas containing alternative functional groups in place of the alkene might also be accommodated by one or more of the identified PSCaa-incorporating synthetase mutants. To test this hypothesis, we assessed the set of previously identified PSCaa-specific synthetase mutants for their ability to incorporate the *meta* substituted azobenzene amino acids Keto-PSCaa **2** (keto substituted) and Cl-PSCaa **3** (benzyl chloride substituted; Figure 1 A).

The keto group in **2** is bioorthogonal and valuable for selective protein modification through reaction with hydrazide or hydroxylamine derivatives.[16] We found that three clones were able to incorporate **2** into GFP by Western blot analysis (Figure S2). The synthetase mutant MmKetoPSCaaRS (Thr302, Ala309, Thr322, Ala346, Gly348, Val401, Trp417, Gly419) was used for determining translation efficiency by co-expression analyses with the Myo_4TAGHis6 gene. Full-length myoglobin was produced only in the presence of 1 mM **2** with good yield (ca. 1.5 mg mL^{-1}), and ESI-FTMS confirmed the site-specific incorporation of **2** at position 4 of myoglobin with high fidelity (Figures S3 and S4).

The benzyl chloride group of Cl-PSCaa **3** may react with the thiol group of a nearby cysteine through proximity-enabled bioreactivity[10] to build a covalent protein bridge in situ. To test the reactivity, we incubated Cl-PSCaa **3** and Boc-protected cysteine at pH 7.5. As monitored by HPLC-MS, the crosslinking between **3** and cysteine was complete after approximately 2 h (Figure S5). We next found that Cl-PSCaa **3** could be genetically incorporated into proteins by the MmPSCaaRS. To investigate whether Cl-PSCaa **3** could be used to build a protein bridge through reacting with a cysteine residue within a protein, we incorporated Cl-PSCaa **3** into the central helix of calmodulin (CaM) at residue 76 and placed cysteine at the i+7 position (residue 83; Figure 5B). By using the tRNA$^{Pyl}_{CUA}$–MmPSCaaRS pair, Cl-PSCaa **3** was incorporated into CaM in *E. coli* to yield 1.2 mg mL^{-1} of CaM after nickel-nitrilotriacetic acid (Ni-NTA) purification (Figure 5A). Mass spectrometry analysis of this purified intact protein indicated that only Cl-PSCaa **3** was incorporated at position 76 and that an intramolecular bond was formed between Cl-PSCaa **3** and the cysteine residue at i+7 position (Figure 5C). A peak at 18069.3 Da was detected, which corresponds to CaM containing the covalent azobenzene bridge. A second peak measured at 17938.2 Da corresponds to CaM containing the covalent bridge and lacking the initiating Met. No peak corresponding to noncrosslinked **3** and cysteine was detected, thus indicating complete bridge formation.

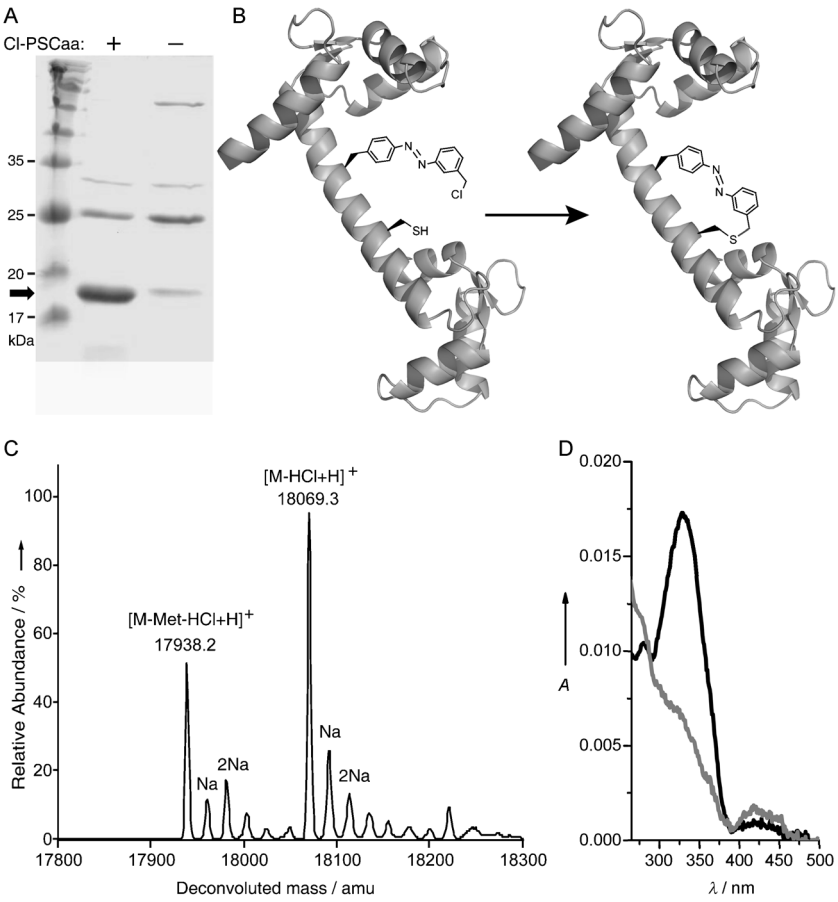

Figure 5. Incorporation of Cl-PSCaa **3** into CaM to form a photoisomerizable covalent protein bridge. A) SDS-PAGE stained with Coomassie shows that tRNA$^{Pyl}_{CUA}$–MmPSCaaRS specifically incorporated Cl-PSCaa **3** into CaM in *E. coli* by suppression of the amber codon introduced at position 76. Samples were normalized for constant cell numbers for each lane. The arrow indicates the position of the full-length protein. B) A scheme showing intramolecular bridge formation in the central helix of CaM through the reaction of Cl-PSCaa **3** with cysteine through proximity-enabled bioreactivity. C) ESI-MS analysis of CaM expressed in the presence of tRNA$^{Pyl}_{CUA}$–MmPSCaaRS supplemented with 1 mM Cl-PSCaa **3**. The crosslinking reaction of Cl-PSCaa **3** with cysteine results in the loss of HCl. Crosslinked products containing the covalent azobenzene bridge: [*M*−HCl+H], expected 18069.8 Da, measured 18069.3 Da; [*M*−Met−HCl+H], expected 17938.6 Da, measured 17938.2 Da. Na^+ adducts of these two species were also detected and labeled. Noncrosslinked products: [*M*+H], expected 18106.2 Da, not detected; [*M*−Met+H], expected 17975.0 Da, not detected. D) UV/Vis spectra of CaM containing the azobenzene bridge before (black line) and after (grey line) light illumination at 365 nm.

To determine whether the azobenzene bridge built in CaM could be photoisomerized, we measured UV/Vis spectra of the purified CaM protein. Before light illumination, the protein showed a strong absorbance at 330 nm and a weak peak at 424 nm, representing π-π* and n-π* transitions, respectively, a pattern characteristic of *trans*-azobenzene (Figure 5D). After illumination at 365 nm, the absorbance at 330 nm decreased along with a concomitant slight increase in the absorbance at 424 nm, thus indicating a clear *trans*-to-*cis* photoisomerization of the azobenzene bridge. To study the effect of bridge photoisomerization on protein conformation, circular dichroism spectra of the bridged CaM were recorded (Figure S6). Consistent with the fact that the *cis* form of PSCaa is less compatible with an i+7 helical spacing than the *trans* form,[7b] *trans–cis* photoisomerization of the azobenzene bridge resulted in a decrease in the helix content of CaM.

In summary, azobenzene-based photoswitchable click amino acids were successfully incorporated into recombinant proteins in *E. coli* and mammalian cells through the use of an expanded genetic code. These unnatural amino acids contain

both a reversible photoswitch and an additional click functional group for further modifications. A covalent protein bridge was spontaneously generated between Cl-PSCaa and an appropriately positioned cysteine in CaM. This bridge photoisomerized upon light activation and altered the protein conformation. Furthermore, the demonstrated promiscuity of the evolved synthetases may allow various alternative azobenzene-based amino acids to be incorporated into proteins. We expect that the genetic incorporation of these photoswitchable click amino acids into proteins will serve as a basis for the design of proteins carrying a photoswitchable bridge, through which protein conformation and bioactivity can be photomodulated in living cells to provide a novel avenue for the minimally invasive investigation of biological processes in vivo.

Received: December 31, 2013
Revised: January 27, 2014
Published online: March 11, 2014

Keywords: amino acids · click chemistry · isomerization · optogenetics · photoswitches

[1] C. Brieke, F. Rohrbach, A. Gottschalk, G. Mayer, A. Heckel, *Angew. Chem.* **2012**, *124*, 8572 – 8604; *Angew. Chem. Int. Ed.* **2012**, *51*, 8446 – 8476.

[2] W. Szymański, J. M. Beierle, H. A. V. Kistemaker, W. A. Velema, B. L. Feringa, *Chem. Rev.* **2013**, *113*, 6114 – 6178 .

[3] for review see A. A. Beharry, G. A. Woolley, *Chem. Soc. Rev.* **2011**, *40*, 4422 – 4437.

[4] for some examples see: a) A. Polosukhina, J. Litt, I. Tochitsky, J. Nemargut, Y. Sychev, I. De Kouchkovsky, T. Huang, K. Borges, D. Trauner, R. N. Van Gelder, R. H. Kramer, *Neuron* **2012**, *75*, 271 – 282; b) A. A. Beharry, L. Wong, V. Tropepe, G. A. Woolley, *Angew. Chem.* **2011**, *123*, 1361 – 1363; *Angew. Chem. Int. Ed.* **2011**, *50*, 1325 – 1327; c) F. Zhang, K. A. Timm, K. M. Arndt, G. A. Woolley, *Angew. Chem.* **2010**, *122*, 4035 – 4038; *Angew. Chem. Int. Ed.* **2010**, *49*, 3943 – 3946; d) C. Hoppmann, P. Schmieder, P. Domaing, G. Vogelreiter, J. Eichhorst, B. Wiesner, I. Morano, K. Rück-Braun, M. Beyermann, *Angew. Chem.* **2011**, *123*, 7841 – 7845; *Angew. Chem. Int. Ed.* **2011**, *50*, 7699 – 7702; e) C. Wyart, F. Del Bene, E. Warp, E. K. Scott, D. Trauner, H. Baier, E. Y. Isacoff, *Nature* **2009**, *461*, 407 – 410; f) R. H. Kramer, J. J. Chambers, D. Trauner, *Nat. Chem. Biol.* **2005**, *1*, 360 – 365; g) D. L. Fortin, M. R. Banghart, T. W. Dunn, K. Borges, D. A. Wagenaar, Q. Gaudry, M. H. Karakossian, T. S. Otis, W. B. Kristan, D. Trauner, R. H. Kramer, *Nat. Methods* **2008**, *5*, 331 – 338; h) A. Cattani-Scholz, C. Renner, C. Cabrele, R. Behrendt, D. Oesterhelt, L. Moroder, *Angew. Chem.* **2002**, *114*, 299 – 302; *Angew. Chem. Int. Ed.* **2002**, *41*, 289 – 292; i) M. Schütt, S. S. Krupka, A. G. Milbradt, S. Deindl, E. K. Sinner, D. Oesterhelt, C. Renner, L. Moroder, *Chem. Biol.* **2003**, *10*, 487 – 490; j) Y. Zhang, F. Erdmann, G. Fischer, *Nat. Chem. Biol.* **2009**, *5*, 724 – 726; M. Volgraf, P. Gorostiza, R. Numano, R. H. Kramer, E. Y. Isacoff, D. Trauner, *Nat. Chem. Biol.* **2006**, *2*, 47 – 52; k) C. Hoppmann, S. Seedorff, A. Richter, H. Fabian, P. Schmieder, K. Rück-Braun, M. Beyermann, *Angew. Chem.* **2009**, *121*, 6763 – 6766; *Angew. Chem. Int. Ed.* **2009**, *48*, 6636 – 6639; l) M. Stein, S. J. Middendorp, V. Carta, E. Pejo, D. E. Raines, S. A. Forman, E. Sigel, D. Trauner, *Angew. Chem.* **2012**, *124*, 10652 – 10656; *Angew. Chem. Int. Ed.* **2012**, *51*, 10500 – 10504; m) C. Hoppmann, C. Barucker, D. Lorenz, G. Multhaup, M. Beyermann, *ChemBioChem* **2012**, *13*, 2657 – 2660.

[5] a) F. Zhang, A. Zarrine-Afsar, M. S. Al-Abdul-Wahid, R. S. Prosser, A. R. Davidson, G. A. Woolley, *J. Am. Chem. Soc.* **2009**, *131*, 2283 – 2289; b) B. Schierling, A.-J. Noel, W. Wende, L. T. Hien, E. Volkov, E. Kubareva, T. Oretskaya, M. Kokkinidis, A. Rompp, B. Spengler, A. Pingoud, *Proc. Natl. Acad. Sci. USA* **2010**, *107*, 1361 – 1366.

[6] for thiol-reactive azobenzene photoswitches see: a) J. R. Kumita, O. S. Smart, G. A. Woolley, *Proc. Natl. Acad. Sci. USA* **2000**, *97*, 3803 – 3808; b) G. A. Woolley, *Acc. Chem. Res.* **2005**, *38*, 486 – 493; c) F. Zhang, O. Sadovski, G. A. Woolley, *ChemBioChem* **2008**, *9*, 2147 – 2154; d) S. Kneissl, E. J. Loveridge, C. Williams, M. P. Crump, R. K. Allemann, *ChemBioChem* **2008**, *9*, 3046 – 3054; e) U. Kusebauch, S. A. Cadamuro, H.-J. Musiol, M. O. Lenz, J. Wachtveitl, L. Moroder, C. Renner, *Angew. Chem.* **2006**, *118*, 7170 – 7173; *Angew. Chem. Int. Ed.* **2006**, *45*, 7015 – 7018; f) U. Kusebauch, S. A. Cadamuro, H.-J. Musiol, L. Moroder, C. Renner, *Chem. Eur. J.* **2007**, *13*, 2966 – 2973; g) L. T. Hien, B. Schierling, A. Yu. Ryazanova, T. S. Zatsepin, E. M. Volkov, E. A. Kubareva, T. I. Velichko, A. Pingoud, T. S. Oretskaya, *Russ. J. Bioorg. Chem.* **2009**, *35*, 549 – 555.

[7] a) C. Hoppmann, P. Schmieder, N. Heinrich, M. Beyermann, *ChemBioChem* **2011**, *12*, 2555 – 2559; b) C. Hoppmann, R. Kühne, M. Beyermann, *Beilstein J. Org. Chem.* **2012**, *8*, 884 – 889.

[8] a) L. Wang, P. G. Schultz, *Angew. Chem.* **2005**, *117*, 34 – 68; *Angew. Chem. Int. Ed.* **2005**, *44*, 34 – 66; b) Q. Wang, A. R. Parrish, L. Wang, *Chem. Biol.* **2009**, *16*, 323 – 336; c) C. C. Liu, P. G. Schultz, *Annu. Rev. Biochem.* **2010**, *79*, 413 – 444.

[9] M. Bose, D. Groff, J. Xie, E. Brustad, P. G. Schultz, *J. Am. Chem. Soc.* **2006**, *128*, 388 – 389.

[10] a) Z. Xiang, H. Ren, Y. S. Hu, I. Coin, J. Wei, H. Cang, L. Wang, *Nat. Methods* **2013**, *10*, 885 – 888; b) I. Coin, V. Katritch, T. Sun, Z. Xiang, F. Y. Siu, M. Beyermann, R. C. Stevens, L. Wang, *Cell* **2013**, *155*, 1258 – 1269; c) Z. Xiang, V. K. Lacey, H. Ren, J. Xu, D. J. Burban, P. A. Jennings, L. Wang, *Angew. Chem.* **2014**, *126*, 2222 – 2225; *Angew. Chem. Int. Ed.* **2014**, *53*, 2190 – 2193.

[11] L. Wang, A. Brock, B. Herberich, P. G. Schultz, *Science* **2001**, *292*, 498 – 500.

[12] J. K. Takimoto, N. Dellas, J. P. Noel, L. Wang, *ACS Chem. Biol.* **2011**, *6*, 733 – 743.

[13] S. W. Santoro, L. Wang, B. Herberich, D. S. King, P. G. Schultz, *Nat. Biotechnol.* **2002**, *20*, 1044 – 1048.

[14] W. Wang, J. K. Takimoto, G. V. Louie, T. J. Baiga, J. P. Noel, K. F. Lee, P. A. Slesinger, L. Wang, *Nat. Neurosci.* **2007**, *10*, 1063 – 1072.

[15] V. K. Lacey, G. V. Louie, J. P. Noel, L. Wang, *ChemBioChem* **2013**, *14*, 2100 – 2105.

[16] a) L. Wang, Z. Zhang, A. Brock, P. G. Schultz, *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 56 – 61; b) H. Liu, L. Wang, A. Brock, C.-H. Wong, P. G. Schultz, *J. Am. Chem. Soc.* **2003**, *125*, 1702 – 1703; c) L. Mahal, K. J. Yarema, C. R. Bertozzi, *Science* **1997**, *276*, 1125 – 1128.

