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Supporting Information

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Mild and Chemoselective Peptide Bond Cleavage of Peptides and Proteins at Azido-homoalanine.

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Synthesis and protein expression: L-azhal (**1**) was synthesized from L-Boc-2,4-diaminobutyric acid (Chem-Impex, Wood Dale, USA) by diazo transfer using Triflic-azide (TfN_3) as described by Lundquist and Pelletier,[1] followed by Boc removal with dioxane/HCl in dichloromethane. After Fmoc reprotection, peptide **P** (sequence: PPHHHHHHPPRGFG**1**GFR was synthesized using standard Fmoc chemistry (Service XS, Leiden, the Netherlands).

To produce **1**-labeled His-tagged recombinant Photoactive Yellow Protein (PYP) from *Ectothiorhodospira halophila* (for sequence see figure 1c), *E coli* strain CAG18491 was transformed with pREP4 and pHISP,[2] and grown in M9 medium containing 400 mg/l **1**, and 50 µg/ml kanamycin. Protein expression was induced by 1 mM IPTG. After lysis, the chromophore p-coumaric acid was inserted,[3] and **AzPYP** was purified on Ni-NTA agarose (Qiagen, Venlo, the Netherlands). Methionine containing His-tagged PYP was produced similarly from transformed *E coli* K12 grown on LB medium.

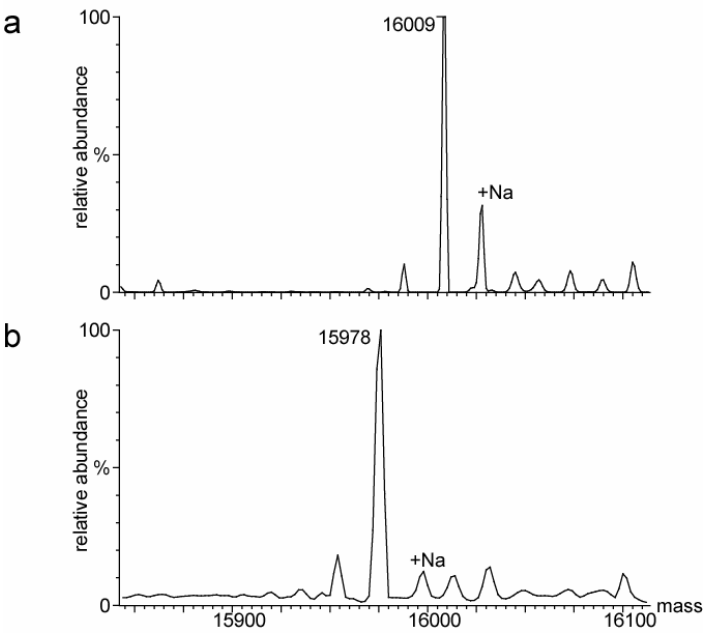
Peptide and protein cleavage with TCEP: cleavage of peptides and protein was achieved in 50 mM Na-acetate buffer pH 5, and up to 100 mM of TCEP, unless otherwise stated. For ^{18}O incorporation, an aliquot of $H_2{}^{18}O$ (>95% atom ^{18}O, Campro Scientific, Veenendaal, The Netherlands) was added so that water with 50% ^{18}O content was obtained. Reactions were left at room temperature overnight.

Homoserine lactones were hydrolyzed by the addition of an excess of unbuffered 1M Na_2CO_3 which was left for 24 h. After vacuum centrifugation, reformation of the lactone was performed in anhydrous TFA for 1h.

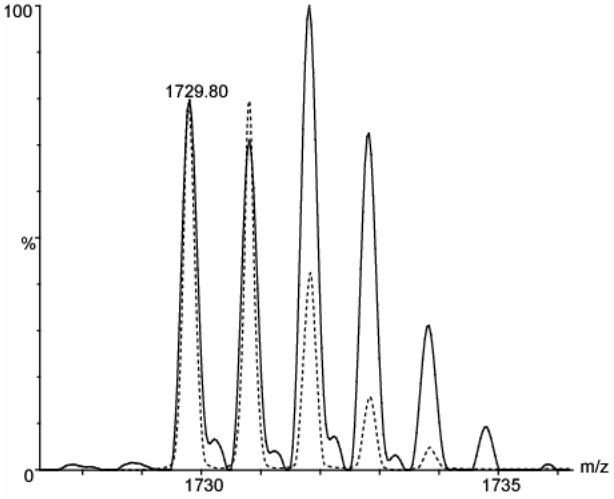
Peptide and protein cleavage with thiols: cleavage of peptides and protein was performed in 100 mM of either 2ME or DTT buffered by Na/Carbonate pH 9.2. To unfold **AzPYP** urea up to 4M (final concentration) was added.

One-pot one stage C terminal elongation experiments were carried out in 100 mM butylamine pH 9. In one pot two stage experiments the peptide was initially incubated in 100 mM NaAc pH5 with 100 mM TCEP, and after 8 h butylamine and NaOH were added so that [$BuNH_2$] was 100 mM and the pH was elevated to 9. Biotin-$(PEO)_4$-amine (Molecular Biosciences, Colorado, USA) present at 100 mM was added to concurrently reducing peptide **P** in 100 mM TCEP in a 100 mM Na/CO_3 buffer, pH 9.2.

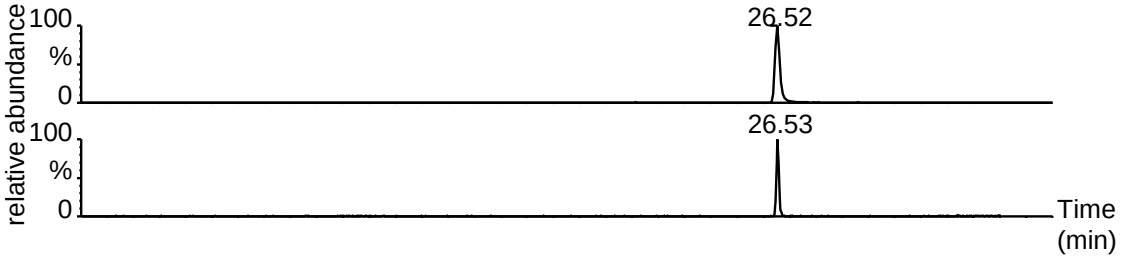
For two step derivatization peptides were harvested by strong cation exchange on Vivaspin S microcentrifuge tubes (Sartorius, Göttingen, Germany) that were eluted with 200 mM Na_2CO_3. The eluate was acidified with TFA, dried in a vacuum centrifuge and the pellet was acidified again with TFA and once more dried.[4] Subsequently, amines were added either neat or dissolved in dry MeOH to the dried lactone peptides.
Methionine containing PYP was cleaved by incubation of 2.5 µg of His-tagged PYP in 20 mg/ml cyanogen bromide in 70% TFA for 2 hours at room temperature.
Prior to mass analysis peptides were desalted with ZipTip C_{18} (Millipore, Bedford, USA), according to the manufacturer's protocol. HPLC separation of cleaved products was performed on a Jupiter Proteo C_{12} column (Phenomenex, Torrance, USA); the gradient of water/acetonitrile being delivered by a SMART system (AmershamPharmacia, Uppsala, Sweden).
Mass spectrometry: Reflectron MALDI-TOF mass spectra were recorded on a Micromass TofSpec 2EC (Micromass, Whyttenshawe, UK). ESI-FTMS and MALDI-FTMS spectra were acquired on a 7T APEX-Q FTMS (Bruker Daltonics, Bremen, Germany) equipped with a CombiSource. For low energy CID the ions were activated in the external collision cell or produced by SORI-CID in the FTMS cell. LCMSMS studies were performed on an Ultimate nano HPLC (LC Packings, Amsterdam, the Netherlands) with a PepMap C_{18} nanocolumn (ID 75µm) coupled to a nano-ESI QTOF mass spectrometer (Micromass). Ions selected for MSMS collided with Argon in the hexapole collision cell.



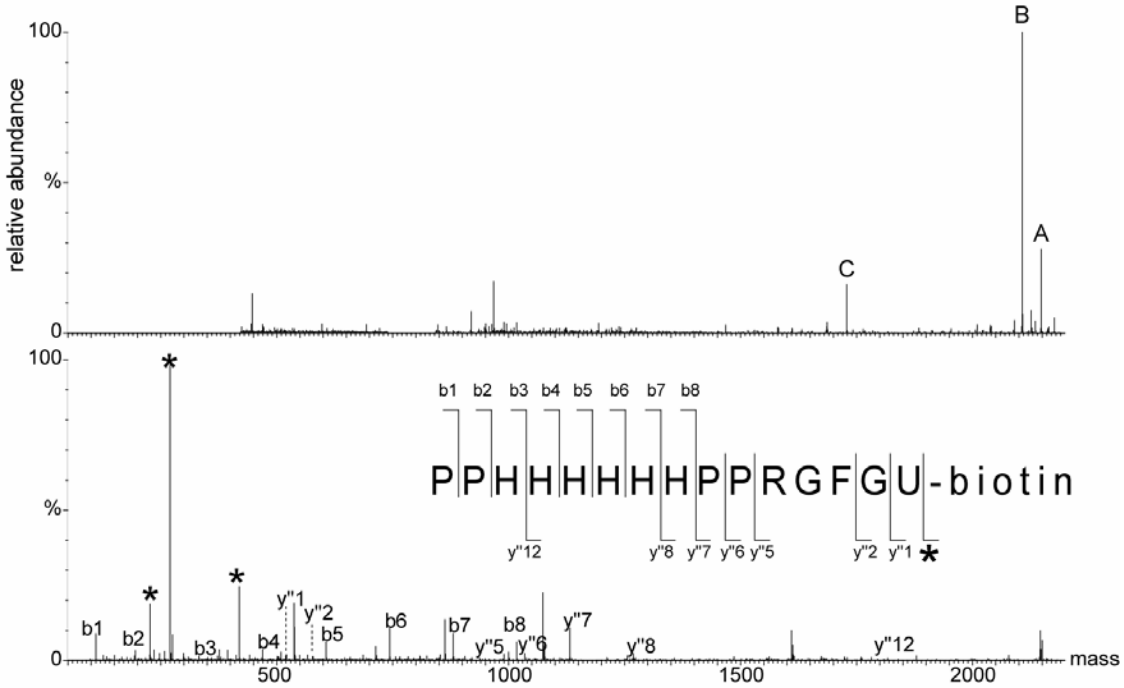
Supplementary figure 1: deconvoluted ESI-QTOF mass spectra of His tagged PYP isolated from pHISP transformed *E coli* CAG18491 after induction with IPTG and reconsitution with the chromophore from a: cells grown on Methionine supplemented media; b: cells grown on media containing **1**. Calculated average masses (MH^+) for His tagged PYP containing 6 × Met or 6 × **1** are 16008.8 and 15978.3 respectively.



Supplementary figure 2: Reductive cleavage by TCEP of peptide **P** performed in 50% ^{18}O enriched water, yielding an isotopic pattern that is indicative of the incorporation of one oxygen atom from water (black trace). The simulated isotope pattern of the peptide with elemental composition $C_{79}H_{104}N_{30}O_{16}$, assuming natural abundance isotopes (dotted trace). A 1:1 ratio of ^{18}O incorporation in the cleavage experiment is clearly visible.



Supplementary figure 3: comparison of the cleavage of **AzPYP** with TCEP and methionine containing PYP with cyanogen bromide. Digests were loaded onto an LCMSMS system (see methods). Extracted ion chromatograms of the doubly charged signal at *m/z* 921.9 of **AzPYP** × TCEP (top trace) and PYP × CNBr (lower trace) show less than 1 sec retention time deviation.



Supplementary figure 4: deconvoluted ESI-MS and MSMS spectra of peptide **P** derivatized with biotinPEO4amine. Peptide **P** was cleaved in the presence of 100 mM biotinamine. Top trace: deconvoluted ESI-MS spectrum reveals components **A**: cleaved and derivatized peptide, **B**: peptide reduced into amine at the position of element **1**, **C**: cleaved peptide that has not been derivatized. Lower trace: MSMS spectrum shows characteristic fragment ions stemming from peptide cleavage (annotated by fragment number; U denotes homoserine) and the biotinPEO4amine moiety (annotated with asterisks *).

Amine	pH [(a)]	Conditions	Homoserine + Lactone	derivatized peptide	Reduced peptide
100 mM $BuNH_2$; present from t=0	9	One pot; one stage	<1%	35%	65%
100mM $BuNH_2$; added after 8h	5; 9	One pot; two stage	20%	15%	65%
$BuNH_2$ (1M in MeOH)	5; na	Two step	10%	50%	40%
$BuNH_2$ (neat; 10.1 M)	5; na	Two step	<1%	60%	40%

Supplementary table 1: yields of one pot and two step derivatizations with butylamine. [(a)]For a two step or two stage derivatization the first pH listed is during TCEP cleavage, the second after addition of $BuNH_2$.

References

[1] J. T. Lundquist, J. C. Pelletier, *Org. Lett.* **2001**, *3*, 781.

[2] R. Kort, W. D. Hoff, M. Van West, A. R. Kroon, S. M. Hoffer, K. H. Vlieg, W. Crielaand, J. J. Van Beeumen, K. J. Hellingwerf, *Embo J* **1996**, *15*, 3209.

[3] J. Hendriks, T. Gensch, L. Hviid, M. A. van Der Horst, K. J. Hellingwerf, J. J. van Thor, *Biophys J* **2002**, *82*, 1632.

[4] J. B. C. Findlay, M. J. Geisow, *Protein sequencing: a practical approach*, IRL press, Oxford, **1989**.