CHEMBIOCHEM

Supporting Information

DOI: 10.1002/cbic.200800816

Supporting Information

for

Protein Iodination by Click Chemistry

Shouliang Dong, Luis Moroder, and Nediljko Budisa*

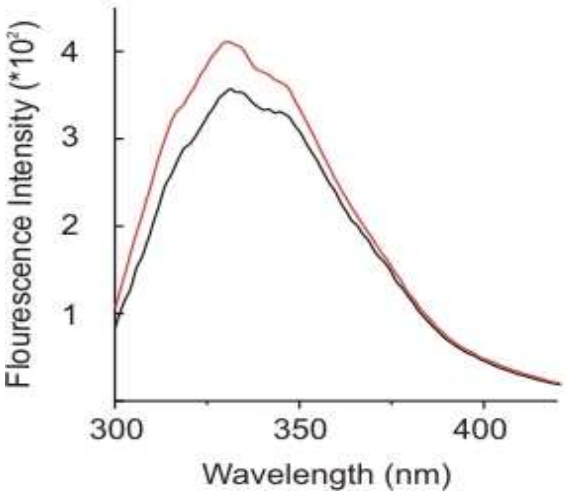

Figure S1. Fluorescence spectra of Hpg-ψ-b* (black) and Hpg(IBA)-ψ-b* (red). Fluorescence emission maxima were measured in the range 300 - 450 nm upon excitation at 280 nm. The concentrations of the samples were 0.5 μM. Note no differences in the position of the emission maxima (λ_{max}=338 nm). The differences in fluorescence intensities are most probably due to the slight differences in sample concentrations caused by pipetting error.

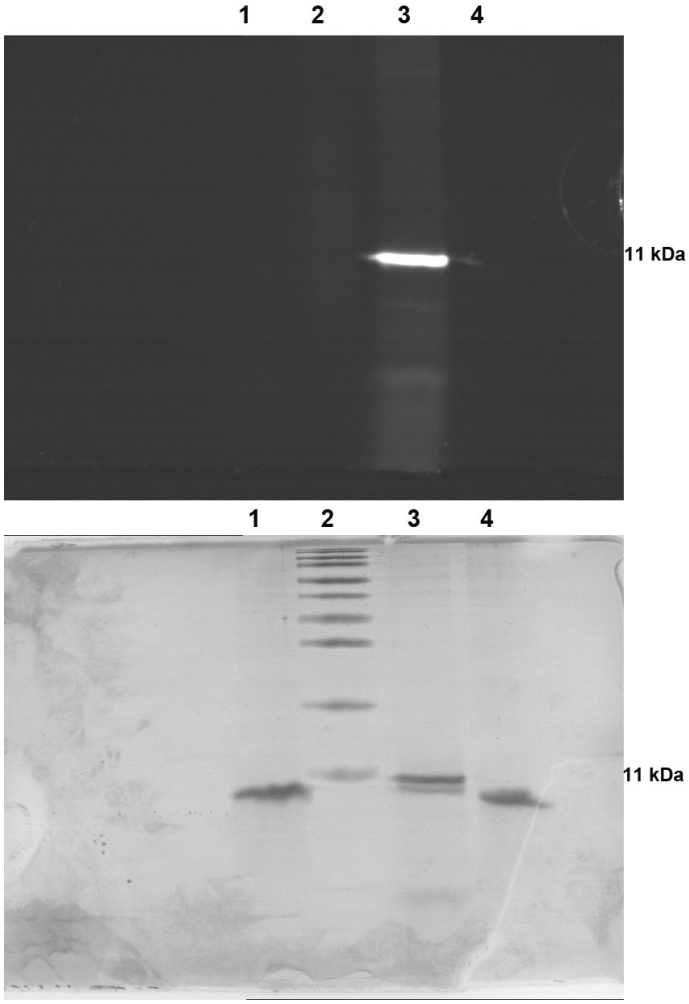

Figure S2. Hpg-ψ-b* labeling with 5-azidofluorescein monitored at 20 % gel SDS-PAGE upon UV exposure (upper gel) and Coomassie staining (lower gel). Lane 1 contains Hpg-ψ-b* and lane 4 lane Met-ψ-b*. In the lane 3 is Hpg-(5-azidofluorescein)-ψ-b*, whereas in lane 2 protein marker ((Fermantas PageRuler™ Prestained Protein Ladder SM0671) is present. Slight shifts in migration height of 5-azidofluorescein-triazole-protein conjugate are visible.

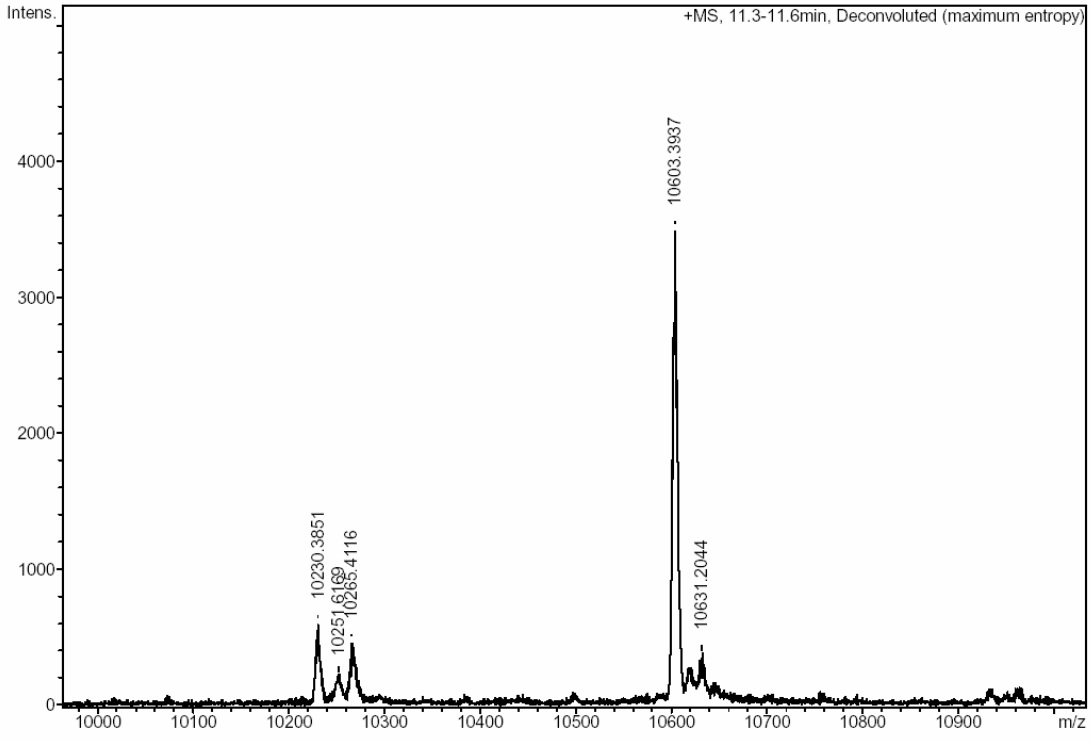

Figure S3. LC-MS of Hpg-ψ-b* before (found: 10230.38, caldc. 10230.65) and after reaction with 5-azidofluorescein (found: 10603.40, calcd. 10603.98). Other minor peaks include the trace presence of unlabelled Met-ψ-b* (10251.6 Da) and of unknown adducts.

Experimental Section

General Methods: High resolution mass spectrometry of protein samples was performed on a micro-TOF-LC mass spectrometer (Bruker Daltonics, Bremen, Germany). Samples were pre-separated by HPLC on a C4 column (Symmetry, Waters, Eschborn, Germany) using a gradient from 90 % A (0.05 % TFA in water) to 90 % B (0.05 % TFA in acetonitrile) within 20 min at a flow rate of 250 µL min^{-1}. The absorbance was detected at 210 nm.

Far-UV CD spectroscopy of protein samples was carried out at 20 ℃ on a JASCO J-715 dichrograph. Protein samples (0.2 mg mL^{-1}) diluted in 1X PBS buffer were measured using quartz Hellma 110-QS cells with 0.1 cm optical path length. Further measurement parameters: speed 50 nm min^{-1}, bandwidth 1 nm, response time 1 s. At least three scans were accumulated in the range 200–250 nm.

Melting curves of Hpg-ψ-b* and Hpg(IBA)-ψ-b* were measured at a concentration of 0.2 mg mL^{-1} by monitoring the changes in residual ellipticity (i.e. unfolding) at 220 nm. The protein solutions were heated from 25 ℃ to 95 ℃ with a rate of 30 ℃ h^{-1} in 110-

QS Hellma quartz cells with an optical path length of 0.1 cm. These experiments were performed on a JASCO J-715 dichrograph equipped with a Peltier-type FDCD attachment (model PFD-350S/350L). At 95 °C and after cooling back to 4 °C CD spectra were measured again to analyze denaturation and renaturation.

Fluorescence spectra of protein samples were measured on a luminescence spectrometer LS 50 B (PerkinElmer Life Sciences; Boston MA) with an excitations/emissions slit of 5 nm. The concentrations of the samples were 0.5 µM. The proteins were excited at 280 nm and the fluorescence was measured in a range of 300 to 500 nm at 20 °C.

D,L-Homopropargylglycine (Hpg): Hpg was synthesized as described before.[1]

5-Azido-fluororescein: To an ice-cold solution of 5-amino-fluororescein (187 mg, 0.54 mmol) in 2 mL CH_3CN was added portionwise *tert*-butylnitrite (96 µL, 0.81 mmol) under stirring followed by NaN_3 (42 mg, 0.65 mmol). The resulting solution was kept at room temperature for 5 h, and the extent of conversion was assessed to be 60 % LC-MS.

Hpg incorporation and purification of Hpg-ψ-b*: The plasmid pQE80ψ-b* for the expression of Aha-ψ-b* was transformed into the Met-auxotrophic *Escherichia coli* strain B834(DE3) (F^-*ompT hsdS*$_D$(r_D^- m_D^-) *gal dcm metE* (DE3)) (Novagen Merck Chemicals Ltd., Nottingham, UK) following standard protocols. The expression of Met→Hpg substituted ψ-b* in inclusion bodies is essentially the same as for Met-containing ψ-b* or Azidohomoalanine-containing ψ-b*.[2] For that reason we followed previously described protocols. Briefly, cells were grown to mid-log phase (OD_{600} 0.6-0.8) in synthetic medium containing 0.025 mM of Met. Met-starved cells were induced for protein expression (IPTG) with concomitant addition of either 50 mg L^{-1} Met or 100 mg L^{-1} of Hpg. The inclusion body isolation and refolding protocol was identical for both variants. Cells were first harvested and lysed in lysis buffer (50 mM Tris-HCl pH 8, 1 mM PMSF) and lysozyme was added. After 30 min at 0 °C the suspension was sonicated and subsequently spun down at 27 000 *g*, 4 °C for 40 min. The pellet with inclusion bodies was resuspended and homogenized in urea buffer (7.5 M urea, 50 mM Tris-HCl pH 8). Insoluble material was removed by centrifugation at 27 000 *g*, 4 °C for 30 min. The supernatant was dialyzed three times against 50 mM Tris-HCl pH 8 and 0.1 mM NaCl at 4 °C. After another spin as described above, the supernatant containing the desired renatured ψ-b* variant and residual cellular proteins was

loaded onto HiTrap Q-sepharose (GE Healthcare Bio-Sciences AB, Uppsala, Sweden), washed with 50 mM Tris-HCl pH 8.0 and eluted with a gradient of 0.1 M NaCl in 50 mM Tris-HCl pH 8.0 to 1 M NaCl in 50 mM Tris-HCl pH 8.0.

References

[1] Dong, S., Merkel, L., Moroder, L. and Budisa, N. (2008). Convenient Syntheses of Homopropargylglycine. *J. Peptide Sci*. **14**, 1148 - 1150.

[2] L. Merkel, Y. Cheburkin, B. Wiltschi, N. Budisa, *ChemBioChem* **2007**, *8,* 2227-2232.