Electronic Supplementary Material (ESI) for Organic & Biomolecular Chemistry

SUPPORTING INFORMATION

Site-specific protein double labeling by expressed protein ligation: applications to repeat proteins

by

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List of Content:

CTPR3:

GNSAEAWYNL GNAYYKQGDY DEAIEYYQKA LELDPNNAEA WYNLGNAYYK QGDYDEAIEY
YQKALELDPN NAEAWYNLGN AYYKQGDYDE AIEYYQKALE LDPNNAEAKQ NLGNAKQKQG

CTPR3[1_3]

GNSAEAWYNL GNAYYKQGDY DEAIEYYQKA LELDP**C**NAEA WYNLGNAYYK QGDYDEAIEY
YQKALELDPN NAEAWYNLGN AYYKQGDYDE AIEYYQKALE LDPN**C**AEAKQ NLGNAKQKQG

CTPR3[1_C]

GNSAEAWYNL GNAYYKQGDY DEAIEYYQKA LELDP**C**NAEA WYNLGNAYYK QGDYDEAIEY
YQKALELDPN NAEAWYNLGN AYYKQGDYDE AIEYYQKALE LDPNNAEAKQ NLGNAKQKQG**C**

CTPR3[2_C]

GNSAEAWYNL GNAYYKQGDY DEAIEYYQKA LELDPNNAEA WYNLGNAYYK QGDYDEAIEY
YQKALELDP**C** NAEAWYNLGN AYYKQGDYDE AIEYYQKALE LDPNNAEAKQ NLGNAKQKQG**C**

CTPR3[N_C]

GCGNSAEAWYNL GNAYYKQGDY DEAIEYYQKA LELDPNNAEA WYNLGNAYYK QGDYDEAIEY
YQKALELDPN NAEAWYNLGN AYYKQGDYDE AIEYYQKALE LDPNNAEAKQ NLGNAKQKQG**C**

Figure S1: Amino acid sequences of the CTPR3 variants. In bold are indicated amino acid mutation/insertion with respect to wtCTPR3.

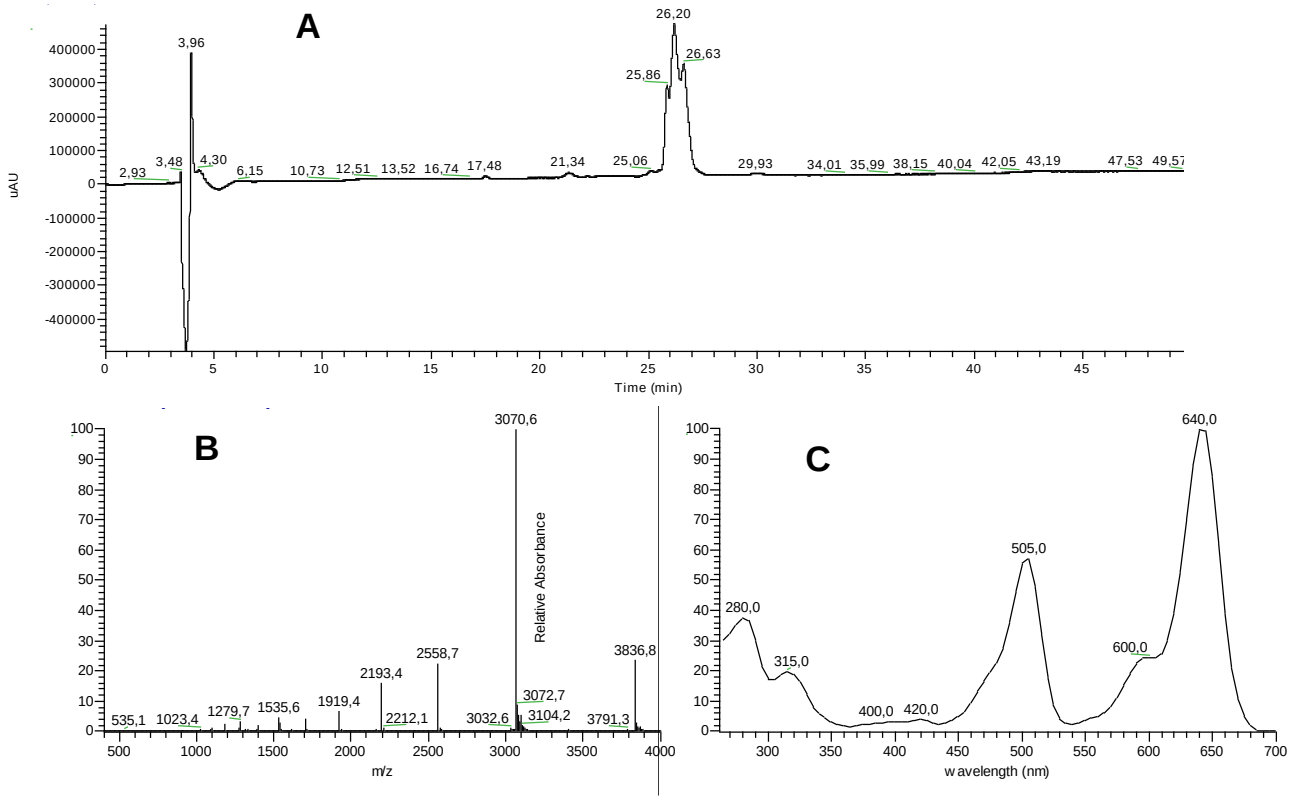

Figure S2. LC-MS analysis of purified double labeled CTPR3[1_3]. A) Chromatographic profile at 210 nm. B) ESI mass spectrum of the chromatographic peaks around 26 min. Experimental molecular mass = 15346.4 Da; calculated molecular mass 15346.8 Da. C) Absorption spectrum of the chromatographic peak around 26 min.

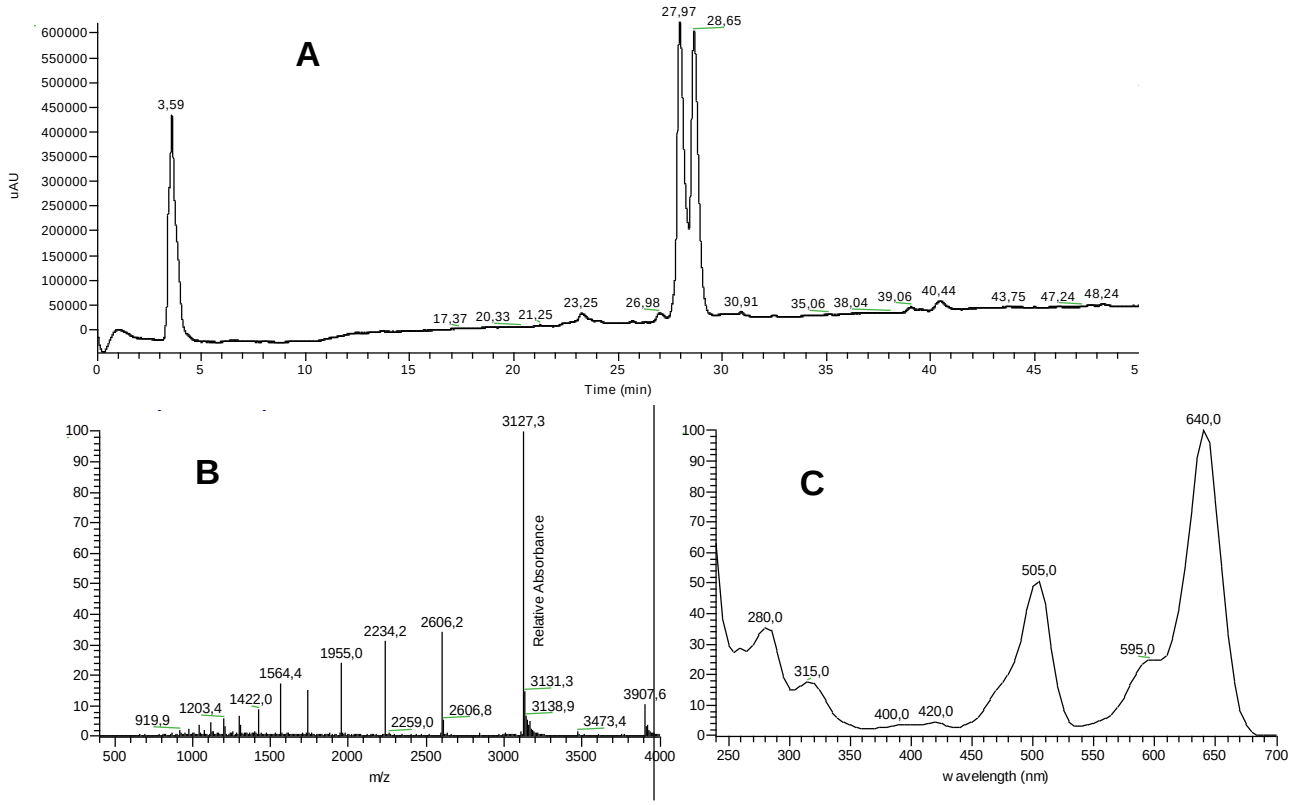

Figure S3. LC-MS analysis of purified doubly-labeled CTPR3[N_C]. A) Chromatographic profile at 210 nm. B) Mass spectrum of the chromatographic peak at 27.97 min. Experimental molecular mass =15631.3 Da; calculated molecular mass =15632.2 Da. Identical mass spectrum is observed for the peak at 28.65 min. C) Absorption spectrum of the chromatographic peak at 27.97 min. Identical spectrum is observed for the peak at 28.65 min.

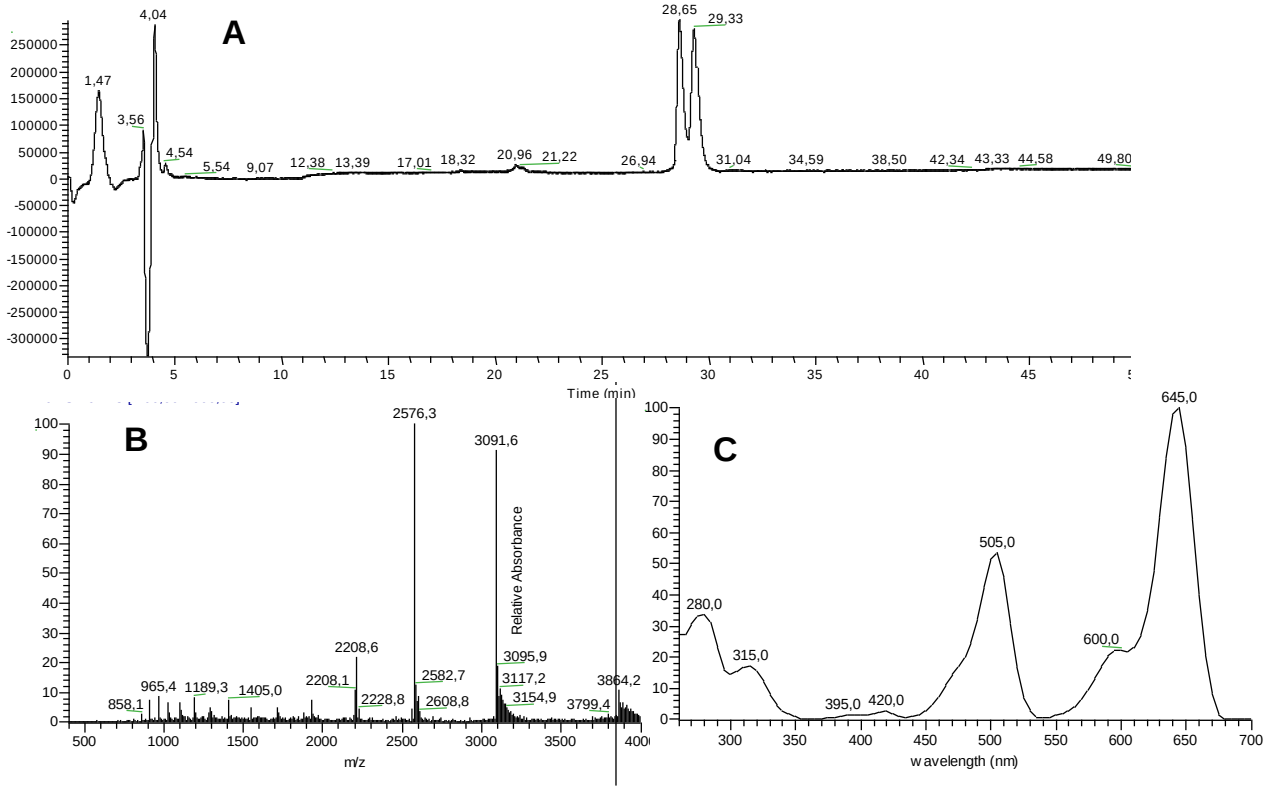

Figure S4. LC-MS analysis of purified doubly-labeled CTPR3[1_C]. A) Chromatographic profile at 210 nm. B) ESI mass spectrum of the chromatographic peak at 28.65 min. Experimental molecular mass =15455.7 Da; calculated molecular mass =15460.9 Da. Identical mass spectrum is observed for the peak at 29.33 min. C) Absorption spectrum of the chromatographic peaks at 28.65 min. Identical spectrum is observed for the peak at 29.33 min.

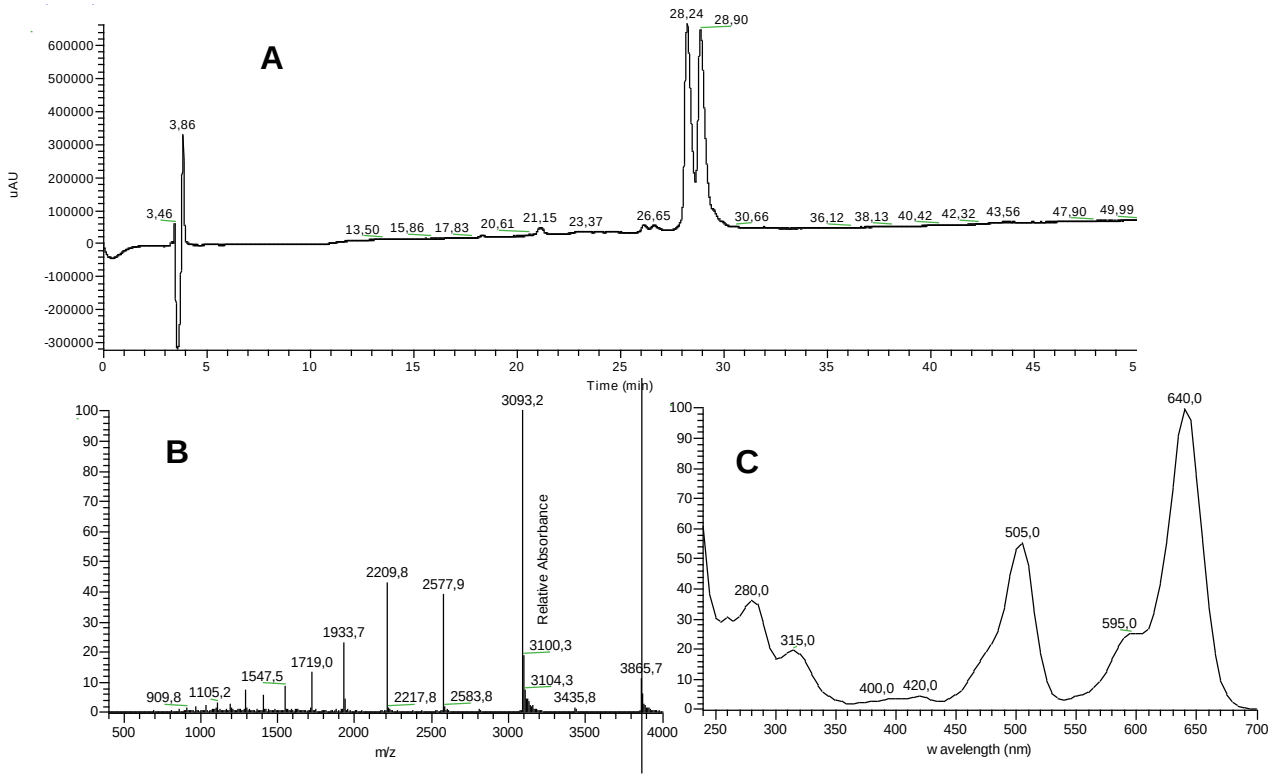

Figure S5. LC-MS analysis of purified doubly-labeled CTPR3[2_C]. A) Chromatographic profile at 210 nm. B) ESI mass spectrum of the chromatographic peak at 28.24 min. Experimental molecular mass =15461.6 Da; calculated molecular mass = 15460.9 Da. Identical mass spectrum is observed for the peak at 28.90 min. C) Absorption spectrum of the chromatographic peak at 28.24 min. Identical spectrum is observed for the peak at 28.90 min.

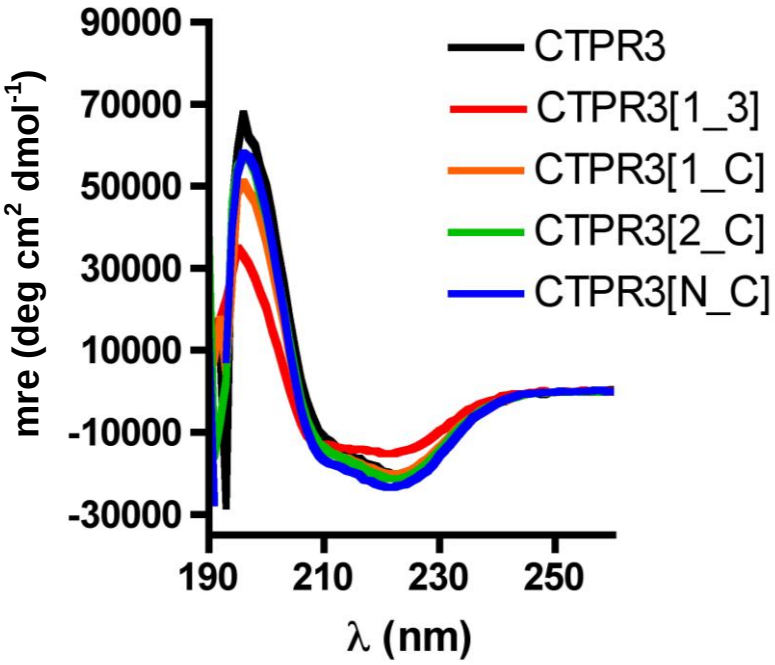

Figure S6. Circular dichroism spectra of CTPR3 variants. Far-uv CD spectrum of CTPR3 (black), and CTPR3 variants CTPR3[1_3] (red), CTPR3[1_C] (orange), CTPR3[2_C] (green), CTPR3[N_C] (blue), doubly labeled with ATTO488 and ATTO 647N dyes. The spectra were acquired at 6 µM protein concentration in 150 mM NaCl, phosphate 50 mM pH 6.5 buffer at 25 °C. Spectra are reported as mean residue ellipticity (mre) deg cm2 dmol-1