Supporting Information for:

Fast photochemical oxidation of proteins (FPOP) maps the topology of intrinsic membrane proteins: light-harvesting complex 2 (LH2) in a Nanodisc

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Analytical Chemistry

Outline

Methods including LH2 Preparation. MSP Preparation Self-assembly of Nanodiscs Steady-state absorption spectroscopy Time-resolved fluorescence spectroscopy, Dynamic light scattering and data analysis.

Figure S1. Steady-state absorption spectrum of LH2 in detergent micelles and Nanodiscs.

Figure S2. Time-resolved fluorescence of LH2. Fluorescence decay of (A) detergent and (B) Nanodisc LH2 preps. Fluorescence was monitored at maximum of the B850 emission band. The fits (red lines) consist of mono-exponential decay function convoluted by instrument response function (IRF). Fluorescence decay lifetimes obtained from data fitting are indicated in graphs.

Figure S3. Dynamic light scattering of empty-nanodisc and LH2-nanodisc

Figure S4. Coverage map of LH2.

Figure S5. Oxidation level of [Leu5]-Enkephalin YGGFL in detergent micelle/Nanodisc environment.

Figure S6. Corresponding amino acids of Mets (*Rb. sphaeroides*) is shown in PDB 1NKZ. Sequence alignment is shown in supplementary figure S7. Met, which corresponds to the N-terminal Met of α subunit from *Rb. sphaeroides* is shown in red; Leu, which corresponds to the transmembrane Met in LH2 from *Rb. sphaeroides*, is shown in yellow; Ala, which corresponds to the N-terminal Met of β subunit from *Rb. sphaeroides*, is shown in blue.

Figure S7. Sequence alignments of LH2. α and β subunits from different LH2 complexes: (1), *Rb. sphaeroides* (2), *Rps. acidophila* (3), *Rs. molischianum*

Figure S8. EIC of m/z = 649.3074 (PAYYQGSAAVAAE). The oxidized residues are marked in red.

Figure S9. MS/MS fragmentation spectrums of different peptides as shown in the picture

Figure S10. Measurement of solvent inaccessible region of LH2 by Pymol. PWL on the C-terminal of β subunit is shown in blue, highly oxidized Pro from α subunit is shown in red.

Figure S11. Oxidation level of N-terminal peptides from both β subunit. The oxidized residues are shown in red. Peptides in detergent are shown in blue, and in Nanodiscs are shown in red.

Methods

LH2 Preparation.

Rb. sphaeroides wild-type strain ATCC 2.4.1 was grown photosynthetically at room temperature (RT) in 1 L bottles, and LH2 was isolated as previously described.[1] Briefly, lauryldimethylamine N-oxide (LDAO, 1.5%) was added to the resuspended membrane pellets with stirring for an hour at RT. After centrifugation, the supernatant was loaded onto an anion-exchange column (QSHP resin, GE Healthcare, Uppsala, Sweden), and a linear gradient elution was performed. The fractions containing LH2 were loaded onto HiLoad Superdex 200 prep grade column (GE Healthcare). The protein-to-pigment ratio was measured from the absorbances at 850 nm (Q_y absorption band of bacteriochlorophyll *a*) and at 280 nm (protein absorption band); fraction showing a ratio greater than 3.0 were collected.

MSP Preparation

A pMSP1E3D1 plasmid was purchased from Addgene (Cambridge, MA). Purification was performed as previously described.[2] Briefly, after breaking the cells by ultra-sonication, the supernatant was loaded onto Ni-NTA agarose column (QIAGEN, Valencia, CA). After extensive washing and elution, the purity of MSP was confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis.

Self-assembly of Nanodiscs

1,2-Dimyristoyl-sn-glycero-3-phosphocholine (DMPC) dissolved in chloroform (Avanti, Alabaster, Alabama) as dispensed into a disposable glass tube and allowed to dry in a fume hood. The tube was placed in a vacuum desiccator overnight to remove residual solvent. A buffer containing cholic acid (Affymetrix, Santa Clara, CA) with a concentration twice that of the lipid was added to the lipid film followed by sonication and gentle heating to solubilize the lipid. The MSP and LH2 were added to the lipid buffer in a 2:1:120 ratio and incubated at room RT for 30 min. After adding amberlite XAD-2 (Sigma Aldrich, St. Louis, MO), the mixture was gently shaken for 2 h at RT until the self-assembly process was complete. Finally, the Nanodisc preparation was purified by HPLC with a Superdex 200 prep grade column (GE Healthcare).[3]

Steady-state absorption spectroscopy

Steady-state absorption spectra of the LH2 were recorded at RT using a Perkin-Elmer Lambda 950 UV-Vis spectrophotometer (Waltham, MA). Prior to measurements the LH2-Nanodisc sample was dissolved in saline buffer (PBS) containing 10 mM phosphate, 140 mM NaCl and 2.3 mM KCl (Sigma Aldrich, St. Louis, MO). For comparison, a solution of free LH2s was dissolved in the same buffer containing 0.02% n-Dodecyl-β-D-Maltopyranoside (DDM).

Time-resolved fluorescence spectroscopy

B850 BChl *a* fluorescence decay dynamics were measured using time-correlated single photon counting (TCSPC) setup based on a stand-alone Simple-Tau 130 system (Becker&Hickl, Germany), equipped with a PMC-100-20 detector (GaAs version with full width at half maximum of instrument response function <200 ps), PHD-400 high speed Si pin photodiode (as triggering module), motorized Oriel Cornerstone 130 1/8 m monochromator with ruled 1200 l/mm grating blazed at 750 nm (Newport, USA), and a manual filter wheel. Excitation pulses at 590 nm (Q_x band of BChl *a*) were delivered by ultrafast optical parametric oscillator Inspire100 (Spectra-Physics, USA) pumped with Mai-Tai, an ultrafast Ti:Sapphire laser (Spectra-Physics, USA), generating ~90 fs laser pulses at 820 nm with a frequency of 80 MHz. The final frequency of the excitation beam was set to 8 MHz (125 ns between excitations) by using a 3980 Pulse Selector from Spectra-Physics. To avoid polarization effects, the excitation beam was depolarized using an achromatic DPU-25 depolarizer (Thorlab, USA). The beam was focused to ~1 mm circular spot at the sample; to assure annihilation-free conditions, the beam intensity was set to ~10^{10} photons/cm^2 per pulse. The signal was collected at right angle to the excitation. The sample absorbances at 850 nm were adjusted to ~0.1.

Dynamic light scattering

The sample was filtered (0.2 μm filter) prior to analysis by dynamic light scattering using a Malvern Zetasizer Nano S/ZS instrument (Worcestershire, UK.) to estimate the diameter of the Nanodisc. The diameter of empty-Nanodisc was also measured as a control at 25 °C. Data were fitted using the Zetasizer software (Worcestershire, UK) to estimate the diameter of the particles.

Data analysis

The oxidation extent was calculated with the equation shown below. Briefly, the strategy relied on an integrated peak area relation between of un-oxidized and oxidized peptides. The integration of peak area was performed by using Xcalibur™ Software. (Thermo Fisher Scientific) For consistency, only +16 modifications were considered in this study as the abundance of di-oxidized products was either low or below the detection limit of the instrument.

$$\textbf{Normalization} = \frac{\textit{Oxidation level of reporter peptide in LH2-Nanodisc}}{\textit{Oxidation level of reporter peptide in LH2-micelle}}$$

$$\textbf{Oxidation level of LH2 peptides in micelle} = \frac{\Sigma I_{ox}}{\Sigma I_{ox} + \Sigma I} \times \textit{Normalization factor}$$

$$\textbf{Oxidation level of LH2 peptides in Nanodisc/reporter peptide} = \frac{\Sigma I_{ox}}{\Sigma I_{ox} + \Sigma I}$$

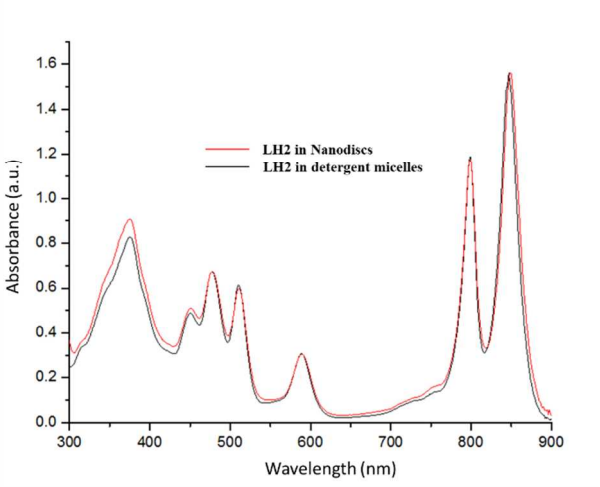


Figure S1. Steady-state absorption spectrum of LH2 in detergent micelles and Nanodiscs.

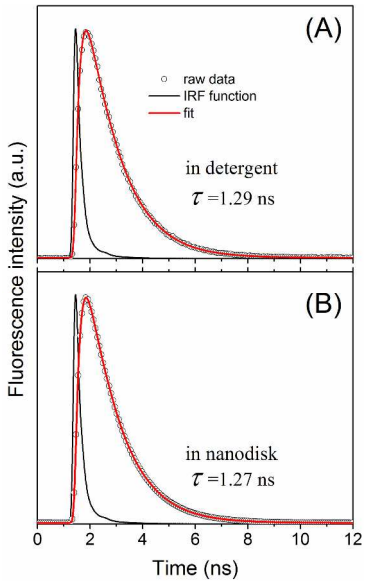
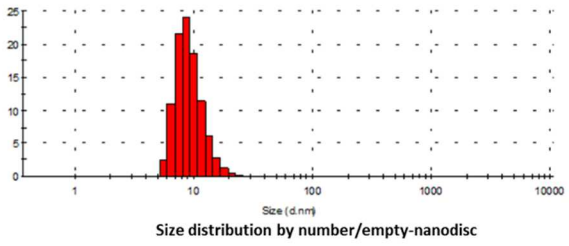
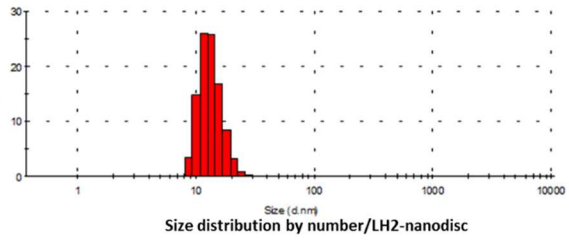


Figure S2. Time-resolved fluorescence of LH2. Fluorescence decay of (A) detergent and (B) Nanodisc LH2 preps. Fluorescence was monitored at maximum of the B850 emission band. The fits (red lines) consist of mono-exponential decay function convoluted by instrument response function (IRF). Fluorescence decay lifetimes obtained from data fitting are indicated in graphs.



Size distribution by number/empty-nanodisc



Size distribution by number/LH2-nanodisc

Figure S3. Dynamic light scattering of empty-nanodisc and LH2-nanodisc

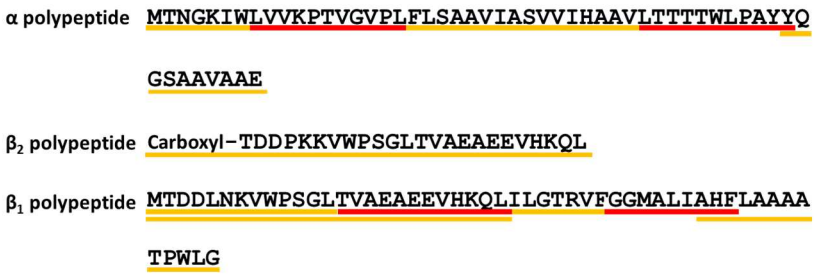


Figure S4. Coverage map of LH2.

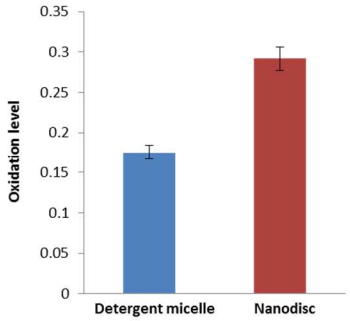


Figure S5. Oxidation level of [Leu5]-Enkephalin YGGFL in detergent micelle/Nanodisc environment.

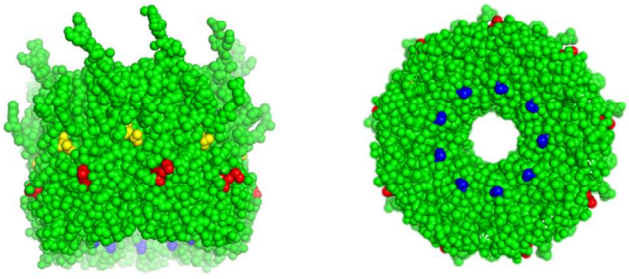


Figure S6. Oxidation level of [Leu5]-Enkephalin YGGFL in detergent micelle/Nanodisc environment.

```
α
1 ---MTNGKIWLVVKPTVGVPLFLSAAVIASVVIHAAVLTTTT--WLPAYYQGSAAVAAE
2 ---MNQGKIWTVVNPAIGIPALLGSVTVIAILVHLAILSHTT--WFPAYWQGGVKKAA-
3 SNPKDDYKIWLVINPSTWLPVIWIVATVVAIAVHAAVLAAPGFNWIAL---GAAKSAAK
      : *** *::*:  :* :   ..: :: :* *:*:     *:     *..  **
β
1 MTDDLNKVWPSGLTVAEAEEVHKQLILGTRVFGGMALIAHFLAAAATPWL-
2 ----------ATLTAEQSEELHKYVIDGTRVFLGLALVAHFLAFSATPWLH
3 -----AERSLSGLTEEEAIAVHDQFKTTFSAFIILAAVAHVLVWVWKPWF-
           : **  ::  :*. .     .*  :* :**.*.   .**:
```

Figure S7. Sequence alignments of LH2. α and β subunits from different LH2 complexes: (1), *Rb. sphaeroides* (2), *Rps. acidophila* (3), *Rs. molischianum*

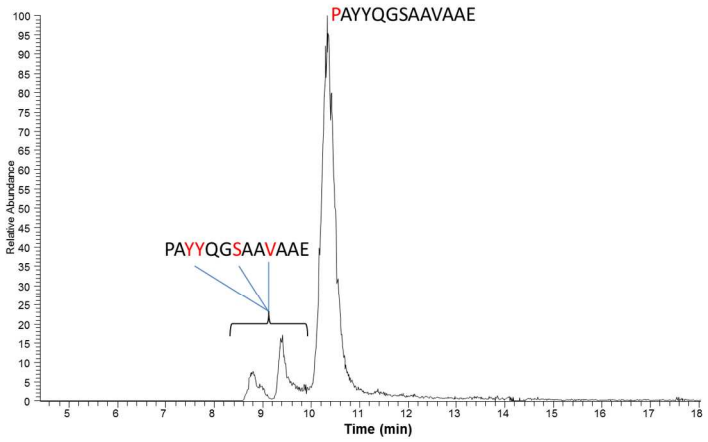
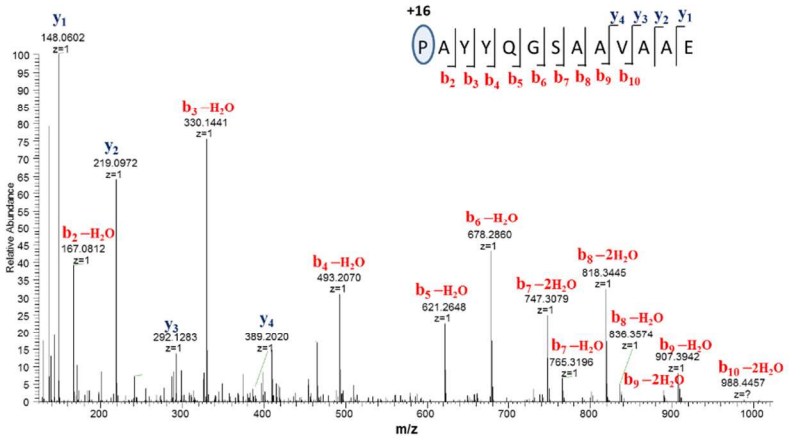


Figure S8. EIC of m/z = 649.3074 (PAYYQGSAAVAAE). The oxidized residues are marked in red.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b3 −H2O 330.1441 z=1
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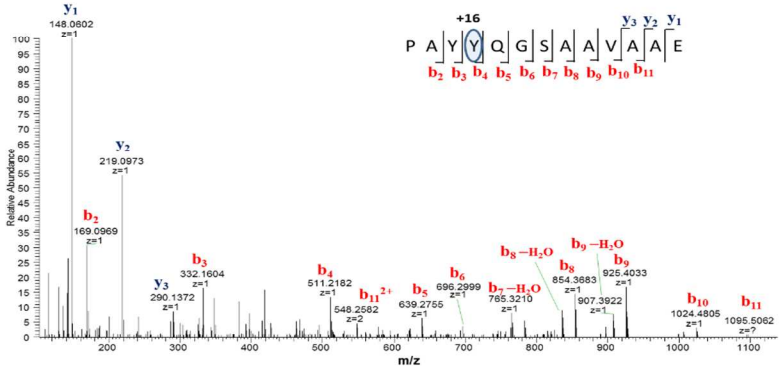
Relative Abundance
m/z

a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b9 −H2O
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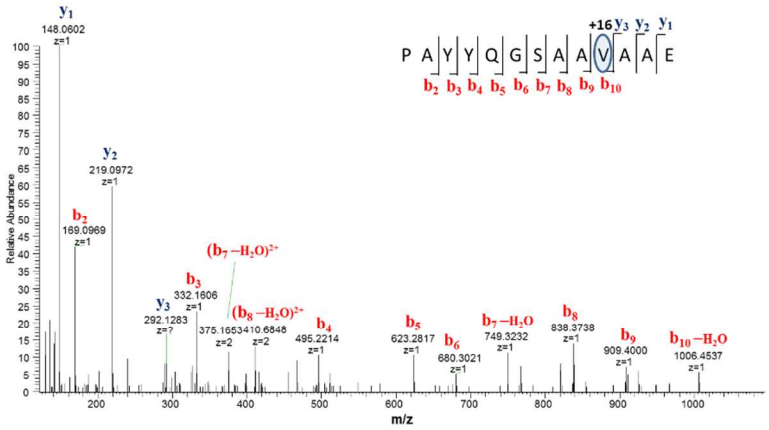
Relative Abundance
m/z

b


+16
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(b7 −H2O)2+
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410.6848 z=2
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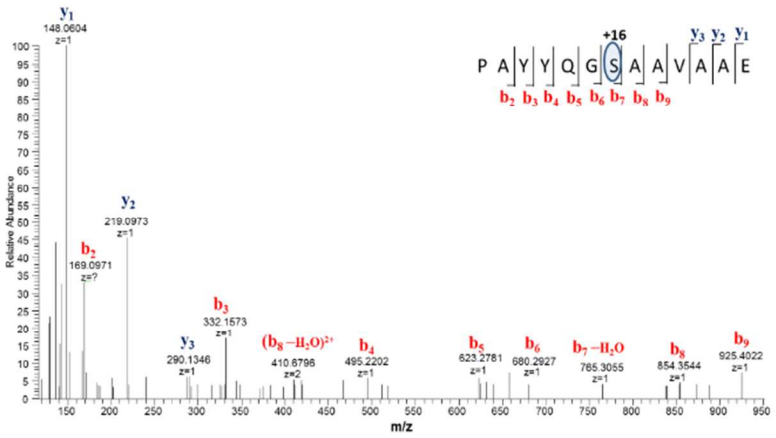
Relative Abundance
m/z

c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290.1346
z=1
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z=2
b4
495.2202
z=1
b5
623.2781
z=1
b6
680.2927
z=1
b7 –H2O
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z=1
b8
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z=1
b9
925.4022
z=1
Relative Abundance
m/z
+16
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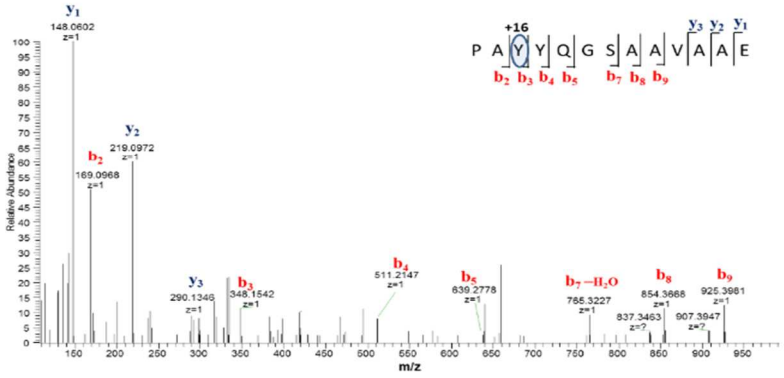
P A Y Y Q G S A A V A A E
b2 b3 b4 b5 b6 b7 b8 b9

d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Relative Abundance
m/z
+16
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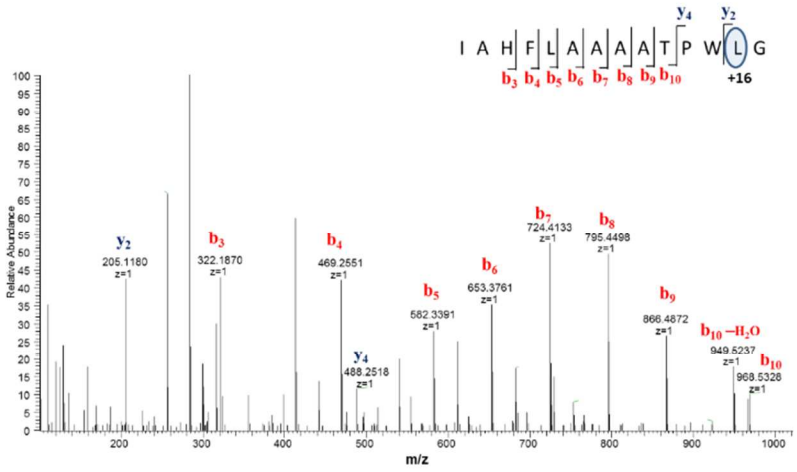
b2 b3 b4 b5 b7 b8 b9

e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b7
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z=1
b8
795.4498
z=1
b9
866.4872
z=1
b10 –H2O
949.5237
z=1
b10
968.5328
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Relative Abundance
m/z
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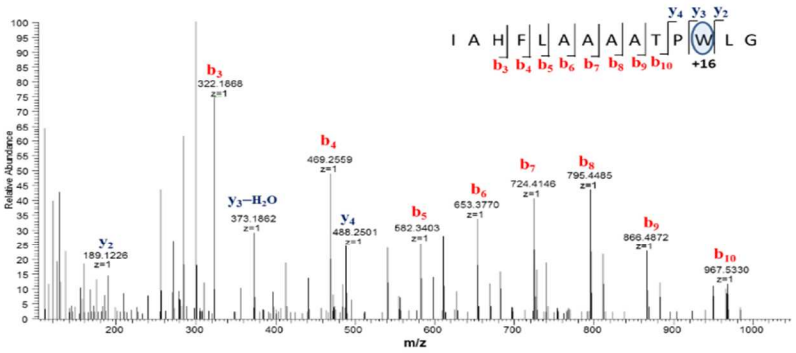
b3 b4 b5 b6 b7 b8 b9 b10
+16

f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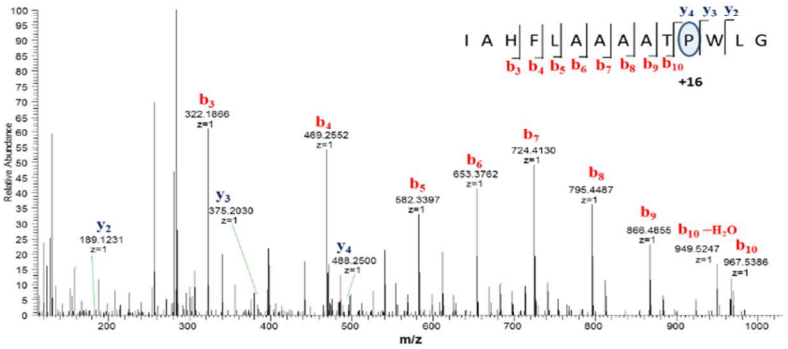
Relative Abundance
m/z

g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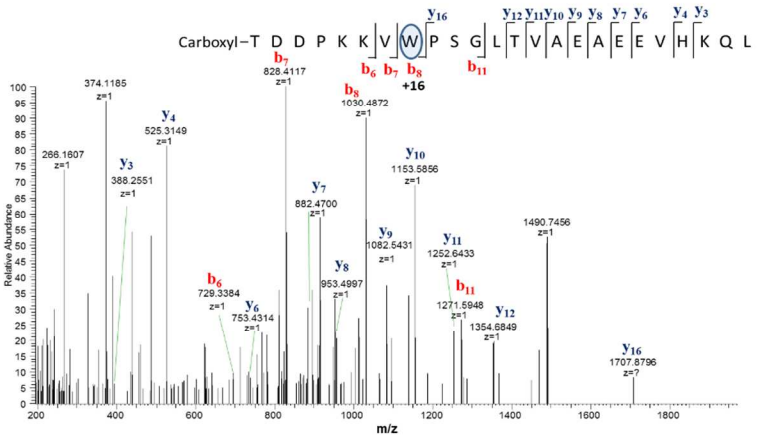
+16
Relative Abundance
m/z

h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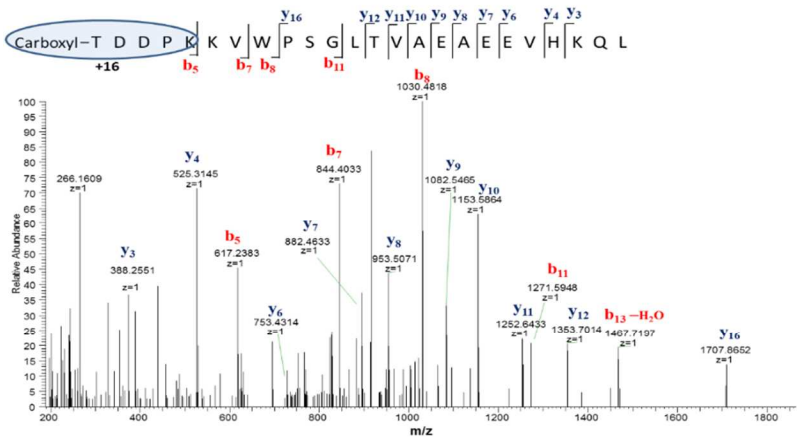
+16
Relative Abundance
m/z

i



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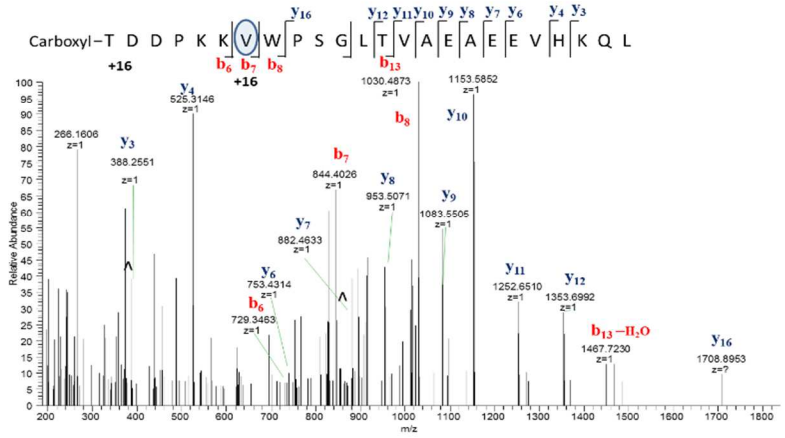

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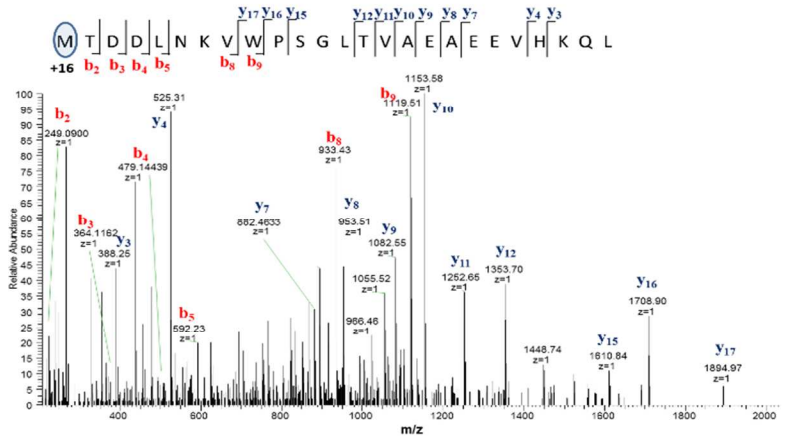

l

Figure S9. MS/MS fragmentation spectrums of different peptides as shown in the picture

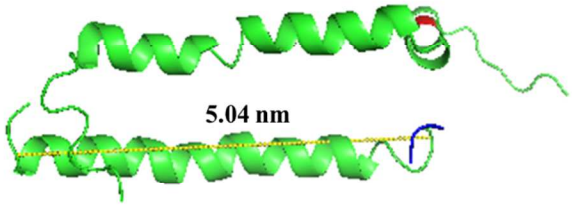

Figure S10. Measurement of solvent inaccessible region of LH2 by Pymol. PWL on the C-terminal of β subunit is shown in blue, highly oxidized Pro from α subunit is shown in red.

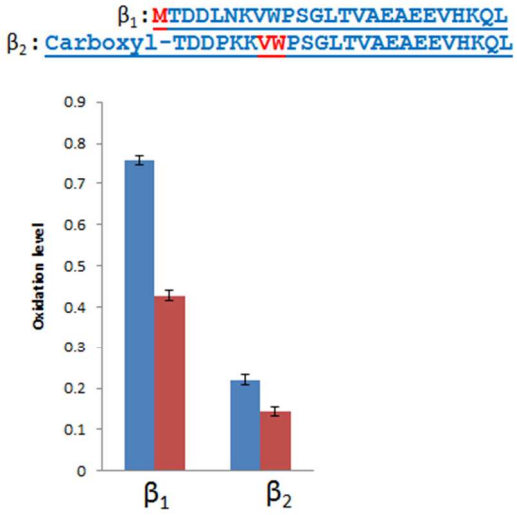

Figure S11. Oxidation level of N-terminal peptides from both β subunit. The oxidized residues are shown in red. Peptides in detergent are shown in blue, and in Nanodiscs are shown in red.

References

1. Lu, Y.; Zhang, H.; Cui, W.; Saer, R.; Liu, H.; Gross, M. L. Blankenship, R. E., *Biochemistry.* **2015,** *54* (49), 7261-7271.
2. Ritchie, T. K.; Grinkova, Y. V.; Bayburt, T. H.; Denisov, I. G.; Zolnerciks, J. K.; Atkins, W. M.; Sligar, S. G. *Method Enzymol.* **2009,** *464*, 211-231.
3. Sligar, S. G. http://sligarlab.life.uiuc.edu/nanodisc/protocols.html.