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^{1}H, ^{15}N, and ^{13}C chemical shift assignments of cyanobacteriochrome NpR6012g4 in the red-absorbing dark state

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Abstract

Cyanobacteriochrome (CBCR) photosensory proteins are phytochrome homologs using bilin chromophores for light sensing across the visible spectrum. NpR6012g4 is a CBCR from *Nostoc punctiforme* that serves as a model for a widespread CBCR subfamily with red/green photocycles. We report NMR chemical shift assignments for both the protein backbone and side-chain resonances of the red-absorbing dark state of NpR6012g4 (BMRB no. 26582).

Keywords

photoreceptor; CBCR; phytochrome; cyanobacteria; tetrapyrrole; NMR

Biological Context

Phytochromes are photosensory proteins utilizing covalently attached linear tetrapyrrole (bilin) chromophores. Light absorption triggers photoisomerization about the 15,16-bilin double bond, allowing the protein to photoconvert between red- and far-red-absorbing states (Auldridge & Forest, 2011). In cyanobacteria, related cyanobacteriochrome (CBCR) sensors provide complete coverage of the spectrum from near-ultraviolet to red (Ikeuchi & Ishizuka, 2008). Phytochromes and CBCRs share a bilin-binding GAF domain with slight structural differences. Several CBCR subfamilies are recognized, including a widespread family exhibiting red/green photocycles in which the red-absorbing dark state interconverts with a green-absorbing photoproduct (Narikawa et al, 2013; Rockwell et al, 2014; Rockwell et al, 2012). The crystal structure of a red/green CBCR, AnPixJg2, was solved recently in the red-absorbing dark state (Narikawa et al, 2013), but presently there is no equivalent information for the photoproduct state. Based on studies of the red/green CBCR NpR6012g4, the blue-shifted chromophore absorption of the photoproduct is thought to arise due to trapping of a twisted chromophore geometry by conserved aromatic residues (Rockwell et al, 2014; Rockwell et al, 2015b). NpR6012g4, the fourth of four GAF domains encoded in tandem by

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the *Npun_R6012* locus of *Nostoc punctiforme*, is 50% identical in sequence to AnPixJg2 (Fig. 1A).

NpR6012g4 switches between a thermally stable red-absorbing dark state and a metastable green-absorbing photoproduct state (Rockwell et al, 2014; Rockwell et al, 2012; Rockwell et al, 2015b). Covalently attached to a conserved cysteine (Cys589) at the bilin A-ring, the phycocyanobilin (PCB) chromophore adopts a C15-*Z, anti* configuration with all 4 NH moieties protonated (Rockwell et al, 2015b). Photoconversion causes formation of the C15-*E,anti* chromophore and places the photoactive D-ring in a hydrophobic environment (Rockwell et al, 2015a). Biological signaling is thought to arise via propagation of these structural changes to adjacent domains, but atomic resolution structures of the same CBCR in both photostates are needed to elucidate such changes. We report detailed NMR resonance assignments for the red-absorbing dark-state of NpR6012g4 as a first step toward achieving this goal.

Methods and Experiments

Expression and Purification of NpR6012g4

The protein sample in this study consists of 180 native residues (M583-G762, Fig. 1) after removal of a C-terminal intein-CBD tag used for affinity purification (Kim et al, 2012). NpR6012g4 was expressed in BL21-AI cells (Invitrogen) grown in M9 minimal media supplemented with ALA (100 μM), ^{15}N-labeled ammonium chloride, and/or ^{13}C-labeled glucose (Cambridge Isotopes) using a published system for induction of protein expression and chromophore biosynthesis (Gambetta & Lagarias, 2001). Affinity purification of NpR6012g4 using a chitin column (NEB) followed our previous procedure (Kim et al, 2012; Rockwell et al, 2012; Rockwell et al, 2015a; Rockwell et al, 2015b). Peak eluted fractions were pooled for overnight dialysis into 10 mM sodium phosphate (pH 7.4) supplemented with 1 mM EDTA to remove residual metal ions followed by final overnight dialysis into 10 mM sodium phosphate (pH 7.4). The protein was concentrated to approximately 0.7 mM, and D_2O was added to 7% (v/v). Dark reversion of the metastable green-absorbing state under these conditions is < 10% after 24 hours at 298 K as reported previously (Rockwell et al, 2015b). All subsequent manipulations were performed in darkness.

NMR spectroscopy

NMR experiments were conducted using Bruker Advance 600 MHz spectrometer equipped with a triple resonance cryogenic probe. All experiments were performed on samples kept in darkness, with spectral acquisition at 298 K. Backbone chemical shift assignments were obtained using ^{1}H,^{15}N-HSQC, HNCA, HNCO, HNCACB, HNCACO, CBCACONH, HBHACONH, ^{1}H,^{15}N-HSQC-TOCSY (mixing time of 60 ms), and ^{1}H,^{15}N-NOESY-HSQC (mixing time of 120 ms) spectra (Ikura et al, 1990). NMR data were processed using NMRPipe (Delaglio et al, 1995) software package and analyzed using SPARKY (www.cgl.ucsf.edu/home/sparky).

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Assignments and Data Deposition

Two-dimensional NMR spectra of the dark state NpR6012g4, ^{15}N-^{1}H HSQC (Fig. 2A) and constant-time ^{13}C-^{1}H HSQC (Fig. 2B) are presented to illustrate representative NMR assignments for backbone and side-chain methyl resonances, respectively. NMR assignments were based on 3D heteronuclear NMR experiments performed on ^{13}C/^{15}N-labeled NpR6012g4 (residues 583–762). The first 16 residues from the N-terminus of NpR6012g4 exhibited weak NMR signals and could not be assigned. These residues comprise a small α-helix found in the AnPixJ crystal structure (Fig. 1). The remaining non-proline residues all exhibited strong backbone amide resonances with uniform intensities, indicative of a well-defined three-dimensional protein structure. More than 90% of the backbone resonances (^{1}HN, ^{15}N, ^{13}Cα, ^{13}Cβ, and ^{13}CO) and 80% of the methyl side-chain resonances are assigned. The chemical shift assignments (^{1}H, ^{15}N, ^{13}C) of NpR6012g4 have been deposited in the BioMagResBank (http://www.bmrb.wisc.edu) under accession number 26582.

We used chemical shift index (Wishart et al, 1992) to assign protein secondary structure for NpR6012g4 (Fig. 1). This secondary structure is broadly similar to that of the closest relative of known structure, the red/green CBCR AnPixJ (Narikawa et al, 2013). However, there are three small differences in secondary structure: (1) the second beta strand in NpR6012g4 is interrupted in the middle by two residues (A636-E637) that form a coil; (2) the fourth helix (α4B) in NpR6012g4 is shorter by three residues; and (3) the fifth beta strand in NpR6012g4 is interrupted by two residues (P704-V705) that form a coil. Using numbering based on AnPixJ, the secondary structure elements of NpR6012g4 are α2: 601–616; β1: 620–625; β2: 635–640; β3A: 644–647; β3B: 654–656; α3A: 658–662; α3B: 667–669; β4: 673–676; α4A: 679–681; α4B: 689–694; β5: 699–707; β6: 710–719; α5: 728–752. The secondary structure differences in β2, α4 and β5 described above for the dark-states of NpR6012g4 versus AnPixJ (Fig. 1A) are predicted to lie close to the chromophore (Narikawa et al, 2013) and might play a functional role in conferring specific target recognition. The most downfield shifted amide resonance is assigned to N720. This residue is predicted in the AnPixJ crystal structure to lie close to the aromatic ring of W643, which may explain the downfield ring current shift. The chemical shift assignments for NpR6012g4 in the green-absorbing photoproduct (reported in a companion paper) will allow us to determine whether these structural changes are general adaptations of NpR6012g4 or if they are specific to the red-absorbing dark state.

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References

Auldridge ME, Forest KT. Bacterial phytochromes: more than meets the light. Crit Rev Biochem Mol Biol. 2011; 46:67–88. [PubMed: 21250783]

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Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeiffer J, Bax A. NMRPipe: a multidimensional spectral processing system based on UNIX pipes. J Biomol NMR. 1995; 6:277–293. [PubMed: 8520220]

Gambetta GA, Lagarias JC. Genetic engineering of phytochrome biosynthesis in bacteria. Proceedings of the National Academy of Sciences of the United States of America. 2001; 98:10566–10571. [PubMed: 11553807]

Ikeuchi M, Ishizuka T. Cyanobacteriochromes: a new superfamily of tetrapyrrole-binding photoreceptors in cyanobacteria. Photochem Photobiol Sci. 2008; 7:1159–1167. [PubMed: 18846279]

Ikura M, Kay LE, Bax A. A novel approach for sequential assignment of 1H, 13C, and 15N spectra of proteins: heteronuclear triple-resonance three-dimensional NMR spectroscopy. Application to calmodulin. Biochemistry. 1990; 29:4659–4667. [PubMed: 2372549]

Kim PW, Freer LH, Rockwell NC, Martin SS, Lagarias JC, Larsen DS. Femtosecond photodynamics of the red/green cyanobacteriochrome NpR6012g4 from Nostoc punctiforme. 1. Forward dynamics. Biochemistry. 2012; 51:608–618. [PubMed: 22148715]

Narikawa R, Ishizuka T, Muraki N, Shiba T, Kurisu G, Ikeuchi M. Structures of cyanobacteriochromes from phototaxis regulators AnPixJ and TePixJ reveal general and specific photoconversion mechanism. Proc Natl Acad Sci USA. 2013; 110:918–923. [PubMed: 23256156]

Rockwell NC, Martin SS, Gulevich AG, Lagarias JC. Conserved phenylalanine residues are required for blue-shifting of cyanobacteriochrome photoproducts. Biochemistry. 2014; 53:3118–3130. [PubMed: 24766217]

Rockwell NC, Martin SS, Lagarias JC. Red/green cyanobacteriochromes: sensors of color and power. Biochemistry. 2012; 51:9667–9677. [PubMed: 23151047]

Rockwell NC, Martin SS, Lim S, Lagarias JC, Ames JB. Characterization of Red/Green Cyanobacteriochrome NpR6012g4 by Solution Nuclear Magnetic Resonance Spectroscopy: A Hydrophobic Pocket for the C15-E, anti Chromophore in the Photoproduct. Biochemistry. 2015a; 54:3772–3783. [PubMed: 25989712]

Rockwell NC, Martin SS, Lim S, Lagarias JC, Ames JB. Characterization of Red/Green Cyanobacteriochrome NpR6012g4 by Solution Nuclear Magnetic Resonance Spectroscopy: A Protonated Bilin Ring System in Both Photostates. Biochemistry. 2015b; 54:2581–2600. [PubMed: 25843271]

Wishart DS, Sykes BD, Richards FM. The chemical shift index: a fast and simple method for the assignment of protein secondary structure through NMR spectroscopy. Biochemistry. 1992; 31:1647–1651. [PubMed: 1737021]

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Fig. 1A

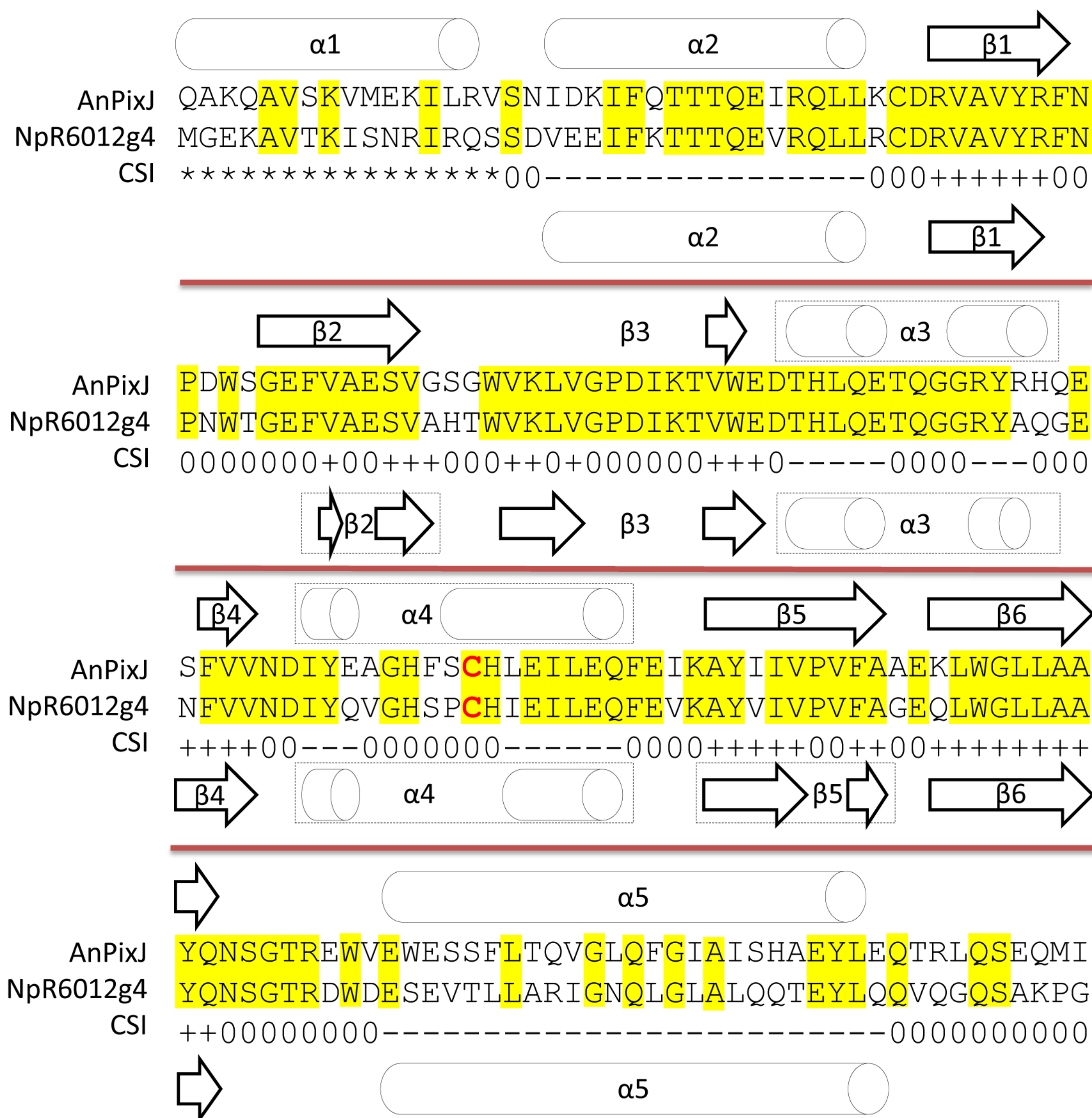

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Fig. 1B

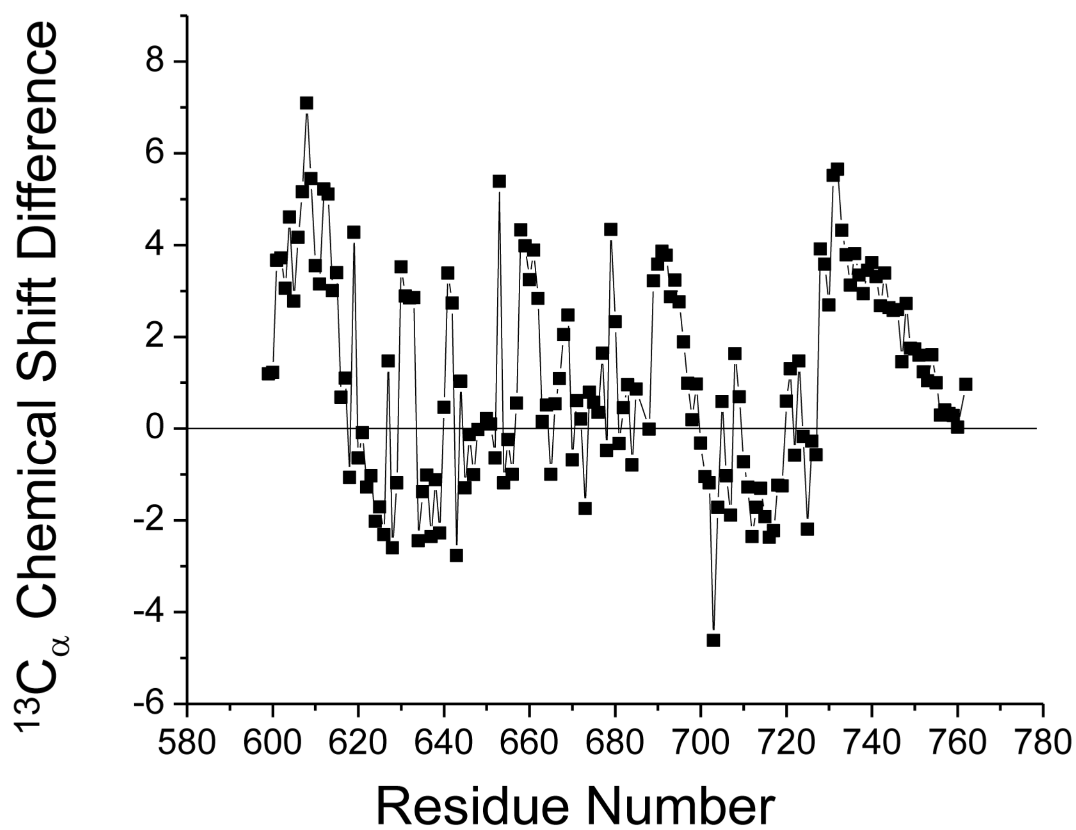

Fig. 1C

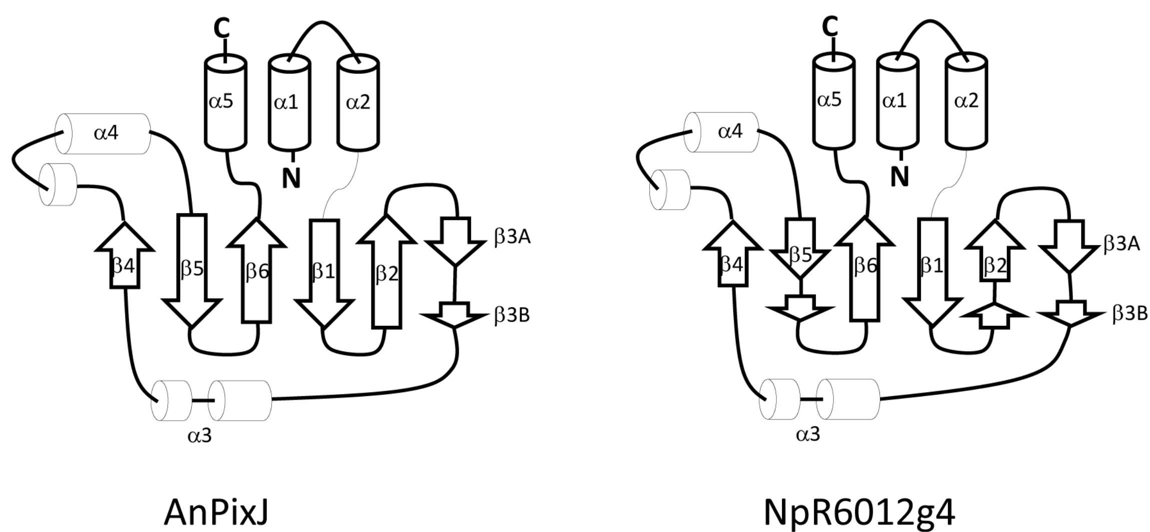

Fig. 1.

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Primary sequence and secondary structure of the red-absorbing dark state of NpR6012g4 compared with those of AnPixJ. (A) Detailed comparison of NpR6012g4 (bottom) and AnPixJ (top). Secondary structural elements for NpR6012g4 were derived from analysis of chemical shift index (Wishart et al, 1992) and sequential NOE patterns. The chemical shift index sign (+, – or 0) for NpR6012g4 is indicated underneath each residue. Unassigned residues are marked with asterisks. Dashed boxes around β2, α3, α4 and β5 highlight structural differences between NpR6012g4 and AnPixJ. (B) Secondary chemical shifts (^{13}Cα) plotted as a function of residue number. (C) Topology diagrams for the CBCR AnPixJ in the red-absorbing dark state (left, (Narikawa et al, 2013) and NpR6012g4 in the red-absorbing dark state (right, this work).

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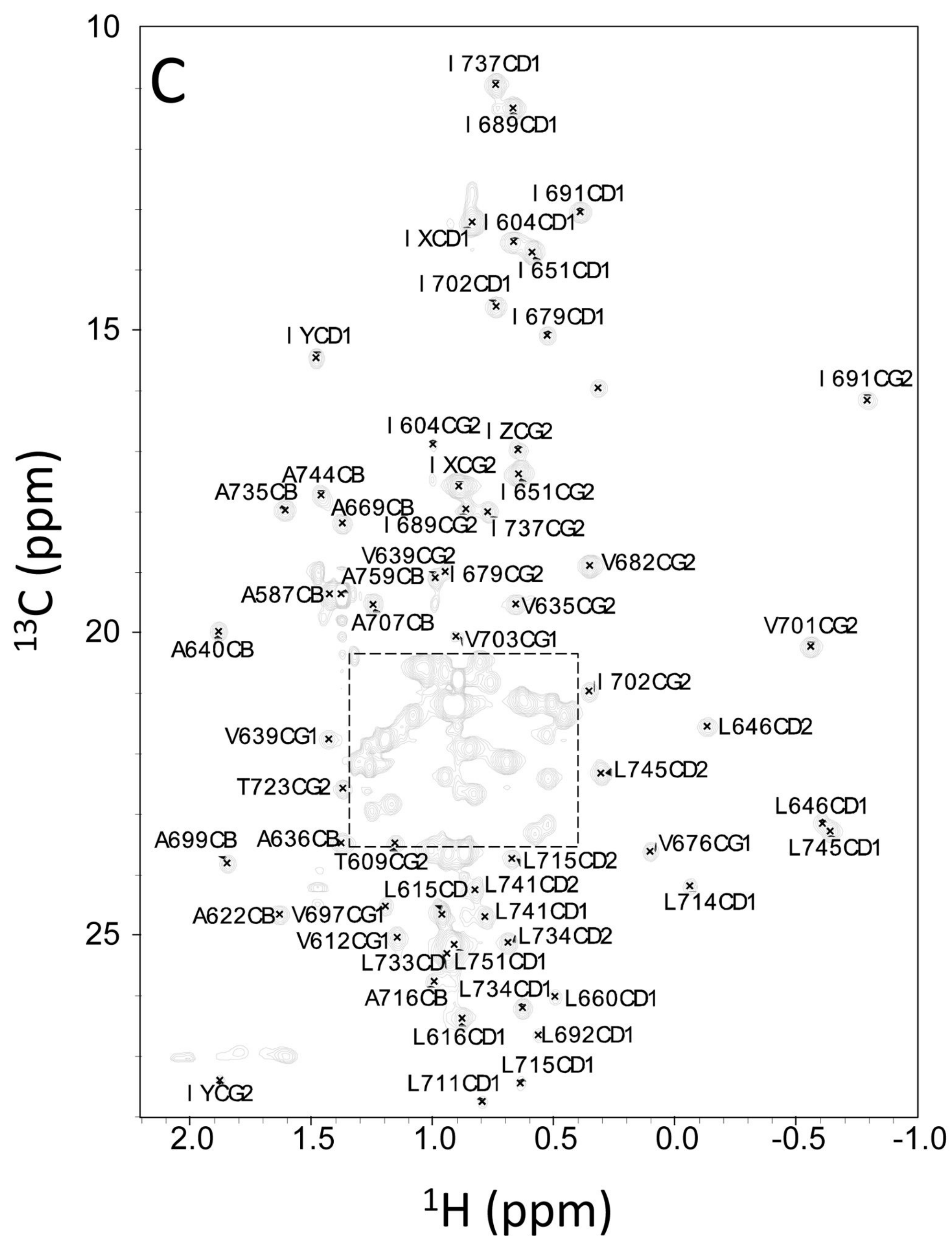

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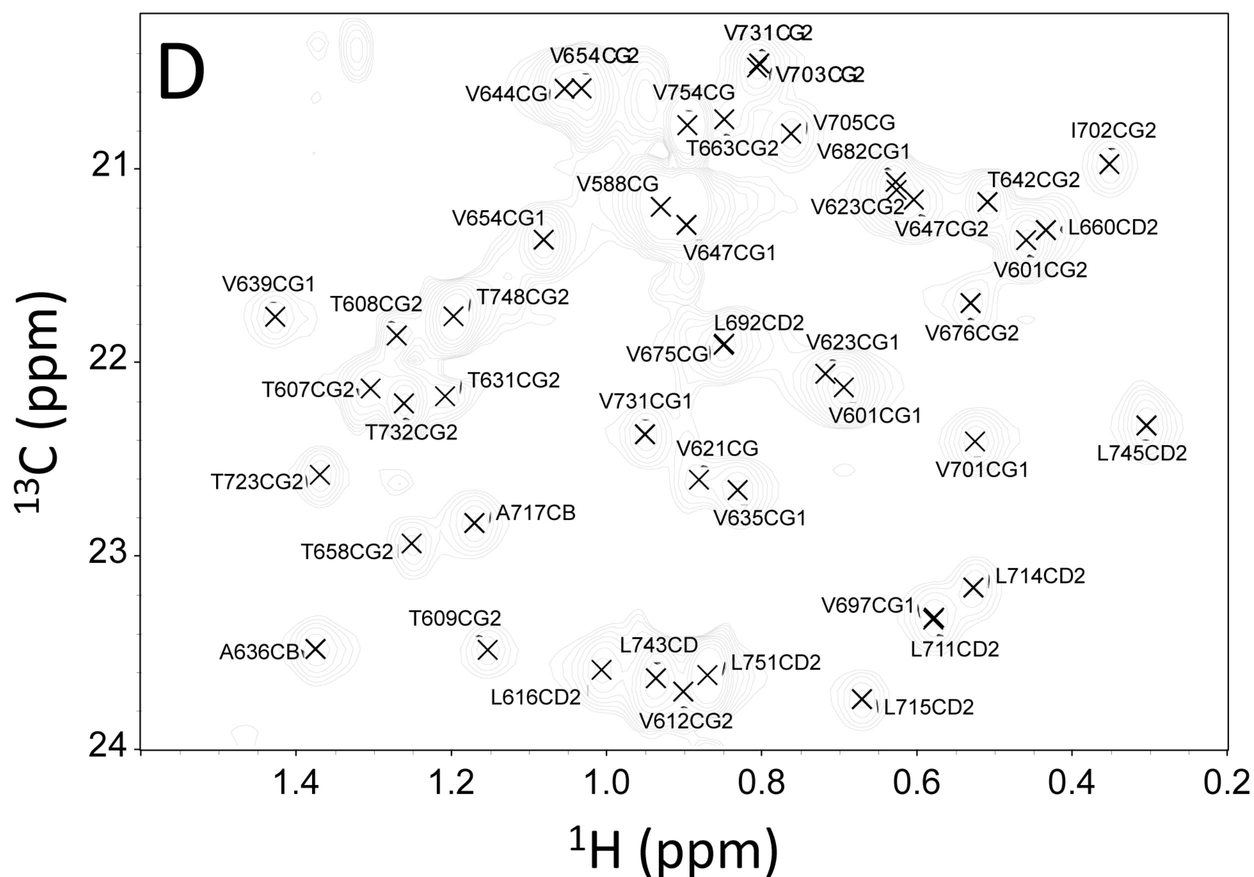

Fig. 2.

Two-dimensional NMR spectra ^{1}H,^{15}N-HSQC (A, B) and ^{1}H,^{13}C-HSQC (C, D) of NpR6012g4 in the red-absorbing dark state recorded at 600 MHz proton frequency. Side chain amide resonances of Asn and Gln are connected with solid lines. Expanded views of the crowded spectral regions (inside the dashed box, panels A and C) are shown in panels B and D. Representative assignments are indicated; complete assignments are available as BMRB accession no. 26582.