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Backbone and side-chain assignments of an effector membrane localization domain from *Vibrio vulnificus* MARTX toxin

Michael C. Brothers¹, Brett Geissler², Grant S. Hisao¹, Brenda A. Wilson³, Karla J.F. Satchell², and Chad M. Rienstra^{1,4,5,*}

¹Department of Chemistry, University of Illinois at Urbana-Champaign Urbana, IL 61801, USA

²Department of Microbiology-Immunology, Northwestern University, Chicago, IL 60611, USA

³Department of Microbiology University of Illinois at Urbana-Champaign Urbana, IL 61801, USA

⁴Department of Biochemistry, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

⁵Center for Biophysics and Computational Biology, University of Illinois at Urbana-Champaign, Urbana, IL 61801, USA

Abstract

¹H, ¹³C, and ¹⁵N chemical shift assignments are presented for the isolated four-helical bundle membrane localization domain from the domain of unknown function 5 (DUF5) effector (MLD_{V_{DUF5}}) of the MARTX toxin from *Vibrio vulnificus* in its solution state. We have assigned 97% of all backbone and side-chain carbon atoms, including 96% of all backbone residues.

Secondary chemical shift analysis using TALOS+ demonstrates four helices that align with those predicted by structure homology modeling using the MLDs of *Pasteurella multocida* toxin (PMT) and the clostridial TcdB and TcsL toxins as templates. Future studies will be towards solving the structure and determining the dynamics in the solution state.

Keywords

Membrane localization domain; four helical bundle; MARTX toxins; *Pasteurella multocida* toxin

Biological Context

The multifunctional autoprocessing repeats-in-toxins (MARTXs) are a class of toxins produced by an array of known bacterial pathogens, including *Vibrio cholerae* and *Vibrio vulnificus*. The hallmark of all MARTX toxins is the presence of a cysteine protease domain that, following translocation into host cells and binding to inositol hexakisphosphate, cleaves the full-length toxin to release effector domains from the central portion of the toxin.

The MARTX toxin encoded by *Vibrio vulnificus* (MARTX_{V_V}) is a 5206 amino acid protein toxin that causes destruction of the intestinal epithelium during infection (Jeong and Satchell, 2012). The toxin carries 5 distinct toxic effector domains that confer a variety of

*Corresponding Author: 217 244-4655 (phone), 217 244-3186 (fax), rienstra@scs.illinois.edu, <http://www.scs.illinois.edu/rienstra/>.

activities to *V. vulnificus* including cytolysis, actin depolymerization, RhoGTPase-inactivation, caspase 3/7-dependent apoptosis, and induction of reactive oxygen species (Satchell 2011). Two of these domains, the Rho-inactivation domain (RID) and domain of unknown function 5 (DUF5) have N-terminal plasma membrane localization domains (MLD), although only the MLD associated with DUF5 (MLD_{V_vDUF5}) is a 8.8 kDa, 79 residue domain that retains full functionality during *in vivo* membrane localization and binding to anionic lipids (Geissler et al. 2010, 2011). Substitution of two basic residues for alanine (K15A or R66A) or a key phenylalanine to asparagine in the L1 region (F16N) significantly reduces phospholipid binding and plasma membrane localization, implicating these as important in membrane association for MLD_{V_vDUF5} (Geissler et al. 2011). Importantly, anionic phospholipids are found in high abundance in the late endosome and in the inner leaflet of the plasma membrane, where DUF5 is presumably exerting its biological function (van Meer et al., 2008).

The conserved region of the MLD is 79 residues; homology models of MLD_{V_vDUF5} have been generated on the basis of crystal structures of related MLDs from *Pasteurella multocida* toxin (2EBF) and the clostridial glucosylating toxins TcdA (3SS1), TcdB (2BVL), and TcsL (2VL8). However, the relatively low sequence identity among the MLDs (often < 50%) would indicate that future studies of MLD_{V_vDUF5} would benefit from experimental data to determine the structures and verify the implied homology. Moreover, NMR studies will help to elucidate the significance of conserved residues, including basic residues such as K15 and R66 as well as hydrophobic residues and helices, including Y25.

Here, we report the resonance assignments of the backbone and sidechain atoms (95 %) of MLD_{V_vDUF5} in solution. Secondary chemical shift analysis using TALOS+ demonstrates that MLD_{V_vDUF5} has similarity to that predicted by homology modeling (Geissler et al. 2010). Resonance assignments will enable studies to determine the mechanism of MLD-membrane association.

Methods and Experiments

Recombinant protein expression and purification

Uniformly labeled ¹³C, ¹⁵N MLD_{V_vDUF5} was expressed in *E. coli* BL21(DE3) cells (Promega, Fitchburg, WI) from the pYCpet plasmid encoding the MLD_{V_vDUF5} gene using previously published conditions (Geissler et al. 2011). Briefly, a 4-mL starter culture in LB was grown at 37°C to an OD₆₀₀ = 2, from which 1 mL of the culture was harvested and resuspended in 1 mL of M9 salt buffer (pH 7.4, 64 mM phosphate, 20 mM KCl, 200 mM NaCl), and then inoculated into a flask containing 200 mL of the labeling medium with ¹³C, ¹⁵N-BioExpress, U-¹³C-glucose, and ¹⁵N-NH₄Cl (Cambridge Isotopes Laboratories, Inc., Andover, MA) (Studier 2005). Specifically, ¹³C, ¹⁵N-BioExpress (10X stock solution) was diluted to 0.1× in M9 minimal media at pH 7.5 containing 2 g/L U-¹³C-glucose and 2 g/L U-¹⁵N NH₄Cl. The culture was grown at 37 °C to an OD₆₀₀ ~0.8. Expression of MLD_{V_vDUF5} was induced with 0.5 mM isopropyl β-D-1-thiogalactopyranoside for 4 h. The resulting labeled protein was isolated and purified as previously published (Geissler et al. 2011). Briefly, the cells were lysed by using sonication in 50 mM Tris-HCl, pH 7.4, containing 500 mM NaCl, and the cell debris pelleted. The

supernatant was purified by Ni²⁺ affinity chromatography (HiPrep 16/160, GE Biosciences, Piscataway, NJ). Protein-containing fractions were concentrated using an Amicon Ultra-15 centrifugal filter unit with MWCO of 3K (Millipore, Billerica, MA), and then separated by Sephadex-S100 size exclusion chromatography using 50 mM Tris-HCl, pH 7.4, with 500 mM NaCl as elution buffer (GE Biosciences, Piscataway, NJ). The protein was then re-concentrated using an Amicon Ultra-2 centrifugal filter with MWCO of 3K (Millipore, Billerica, MA). This resulted in >99% purity based on SDS-PAGE analysis with a yield of 40–50 mg/L of 1 mM U-¹³C, ¹⁵N-labeled MLD_{V_VDUF5} in 50 mM Tris-HCl, pH 7.4, with 500 mM NaCl, 10% D₂O (v/v) and 0.01% 4,4-dimethyl-4-silapentane-1-sulfonic acid.

NMR spectroscopy

Solution NMR spectra were acquired at the School of Chemical Sciences NMR Facility (University of Illinois at Urbana-Champaign) on a Varian INOVA 600 spectrometer equipped with a 5 mm, triple resonance (¹H-¹³C-¹⁵N) triaxial gradient probe, using VNMRJ version 2.3 with the BioPack suite of pulse programs. Spectra of the U-¹³C, ¹⁵N MLD_{V_VDUF5} samples were acquired at 30°C. 2D ¹H-¹⁵N HSQC spectra were collected before and after the spectra used for assignments, consisting of the standard suite of gradient-based heteronuclear triple resonance 3D spectra (HNCO, HN(CA)CO, HNCACB, CBCA(CO)NH) (Clubb et al. 1992; Kay et al. 1990; Grzesiek and Bax 1992b; Grzesiek and Bax 1992a). ¹³C-HCCH-TOCSY, CC(CO)NH, and ¹³C-NOESY-HSQC (Bax et al. 1990; Grzesiek et al. 1993; Marion et al. 1989) were acquired and analyzed to obtain sidechain resonance assignments. Spectra were processed using NMRPipe (Delaglio et al. 1995) and analyzed in Sparky (Goddard and Kneller 2006).

Extent of assignments and data deposition

The data sets enabled assignments of ~95% of the H_N, N, C_α, C_β, C' and carbon side-chain assignments of MLD_{V_VDUF5}, including 96% of the backbone resonances. We have used the same naming convention as the crystal structure (PDB ID 4ERR). The assignments are deposited in the BMRB under accession number 18562. The ¹H-¹⁵N gradient HSQC spectrum (Fig. 1) demonstrates good resolution and dispersion, consistent with a uniform, folded structure. We do not observe amide ¹H-¹⁵N correlations in the HSQC for the following residues: Q3, F17, R41, T63, G65, R66, and Q68. Of the backbone amides signals observed, all but one were assigned; the missing assignment likely arises from the C-terminal hexahistidine-tag. In most cases, we were able to assign the CA, CB, and C' of the residues missing amide frequencies in the HSQC using CBCA(CO)NH and HNCO experiments. C(CO)NH and ¹³C-edited HSQC-TOCSY experiments enabled us to assign the ¹³C side-chain resonances. In these experiments, we observe significant degeneracy in many of the aliphatic residues for the CD of leucines 29, 32, 34, 50, 70, 73, 81, and 83 as well as the CG in V16.

TALOS+ (Shen et al. 2009) analysis demonstrates four helices, consistent with those observed in the homology model of MLD_{V_VDUF5}, based on structure prediction from the crystal structure of PMT (Fig. 2). The helices consist of residues 7–15 (H1), 23–36 (H2), 40–61 (H3), and 67–81 (H4), with intervening loop residues 16–22 (L1), 37–39 (L2), and 62–66 (L3). The missing assignments of non-proline amide resonances belong to the N-

terminal methionine, the C-terminal hexahistidine-tag, a single residue in the first turn of H3 (R41), and residues in L1 (F17) and L3 (T63, G65) or the first turn of H4 (R66, Q68). This suggests amide exchange in these regions, which indicates an exposure to solution, as would be expected in loop regions or solvent exposed helices.

According to structure prediction, loops L1 and L3 are adjacent to each other at the same end of the 4HBM. Chemical exchange broadening is observed throughout these regions, which may be key for membrane association (Geissler et al. 2010; Geissler et al. 2011). Resonance assignments provide a basis for solving the structure of the isolated MLD_{VvDUF5} and understanding the conformation and dynamics of membrane association.

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Abbreviations

DUF	Domain of unknown function
MARTX	Multifunctional autoprocessing repeats-in-toxins
MLD	Membrane localization domain
MLD_{PMT}	Membrane localization domain from <i>Pasteurella multocida</i> toxin
MLD_{VvDUF5}	Membrane localization domain from MARTX toxin from <i>Vibrio vulnificus</i>
PMT	<i>Pasteurella multocida</i> toxin

References

- Bax A, Clore GM, Gronenborn AM. ¹H-¹H Correlation Via Isotropic Mixing of ¹³C Magnetization, a New 3-Dimensional Approach for Assigning ¹H and ¹³C Spectra of ¹³C-Enriched Proteins. *J Magn Reson.* 1990; 88(2):425–431.
- Clubb RT, Thanabal V, Wagner G. A Constant-Time 3-Dimensional Triple-Resonance Pulse Scheme to Correlate Intraresidue ¹H(N), ¹⁵N, and ¹³C (') Chemical-Shifts in ¹⁵N-¹³C-Labeled Proteins. *J Magn Reson.* 1992; 97(1):213–217.
- Delaglio F, Grzesiek S, Vuister GW, Zhu G, Pfeifer J, Bax A. NMRpipe - a Multidimensional Spectral Processing System Based On Unix Pipes. *J Biomol NMR.* 1995; 6(3):277–293. [PubMed: 8520220]
- Geissler B, Ahrens S, Satchell KJF. Plasma membrane association of three classes of bacterial toxins is mediated by a basic-hydrophobic motif. *Cell Microbiol.* 2011; 14(2):286–298. [PubMed: 22044757]
- Geissler B, Tungekar R, Satchell KJF. Identification of a conserved membrane localization domain within numerous large bacterial protein toxins. *P Natl Acad Sci USA.* 2010; 107(12):5581–5586.
- Goddard, TD.; Kneller, DG. *Sparky 3.* 3.106 edn. San Francisco: University of California; 2006.
- Grzesiek S, Anglister J, Bax A. Correlation of Backbone Amide and Aliphatic Side-Chain Resonances in ¹³C/N-15-Enriched Proteins by Isotropic Mixing of ¹³C Magnetization. *J Magn Reson Ser B.* 1993; 101(1):114–119.
- Grzesiek S, Bax A. Correlating Backbone Amide and Side-Chain Resonances in Larger Proteins by Multiple Relayed Triple Resonance NMR. *J Am Chem Soc.* 1992a; 114(16):6291–6293.
- Grzesiek S, Bax A. An Efficient Experiment for Sequential Backbone Assignment of Medium-Sized Isotopically Enriched Proteins. *J Magn Reson.* 1992b; 99(1):201–207.

- Kamitani S, Kitadokoro K, Miyazawa M, Toshima H, Fukui A, Abe H, Miyake M, Horiguchi Y. Characterization of the Membrane-targeting C1 Domain in *Pasteurella multocida* Toxin. *J Biol Chem*. 2010; 285(33):25467–25475. [PubMed: 20534589]
- Kay LE, Ikura M, Tschudin R, Bax A. 3-Dimensional Triple-Resonance NMR Spectroscopy of Isotopically Enriched Proteins. *J Magn Reson*. 1990; 89(3):496–514.
- Kitadokoro K, Kamitani S, Miyazawa M, Hanajima-Ozawa M, Fukui A, Miyake M, Horiguchi Y. Crystal structures reveal a thiol protease-like catalytic triad in the C-terminal region of *Pasteurella multocida* toxin. *P Natl Acad Sci USA*. 2007; 104(12):5139–5144.
- Marion D, Driscoll PC, Kay LE, Wingfield PT, Bax A, Gronenborn AM, Clore GM. Overcoming the Overlap Problem in the Assignment of ^1H -NMR Spectra of Larger Proteins by Use of 3-Dimensional Heteronuclear ^1H - ^{15}N Hartmann-Hahn Multiple Quantum Coherence and Nuclear Overhauser Multiple Quantum Coherence Spectroscopy - Application to Interleukin-1-Beta. *Biochemistry*. 1989; 28(15):6150–6156. [PubMed: 2675964]
- Satchell KJF. Structure and Function of MARTX Toxins and Other Large Repetitive RTX Proteins. *Annu Rev Microbiol*. 2011; 65:71–90. [PubMed: 21639783]
- Shen Y, Delaglio F, Cornilescu G, Bax A. TALOS+: a hybrid method for predicting protein backbone torsion angles from NMR chemical shifts. *J Biomol NMR*. 2009; 44(4):213–223. [PubMed: 19548092]
- Studier FW. Protein production by auto-induction in high density shaking cultures. *Protein Express Purif*. 2005; 41(1):207–234.
- Wilson BA, Ho MF. Cellular and molecular action of the mitogenic protein-deamidating toxin from *Pasteurella multocida*. *FEBS J*. 2011; 278(23):4616–4632. [PubMed: 21569202]
- van Meer G, Voelker DR, Feigenson GW. Membrane lipids: where they are and how they behave. *Nat Rev Mol Cell Bio*. 2008; 9(2):112–124. [PubMed: 18216768]

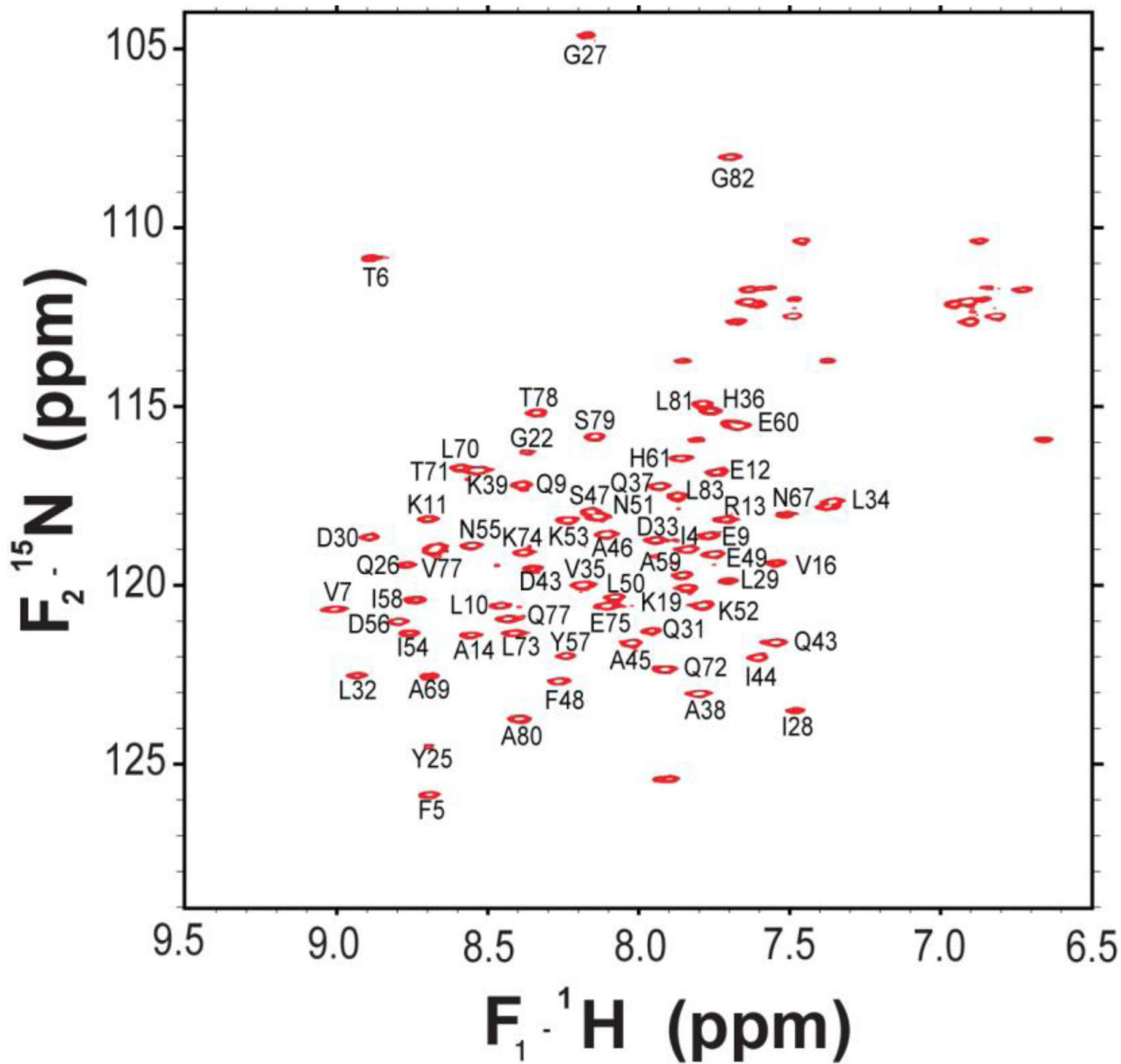


Figure 1.

${}^1\text{H}$ - ${}^{15}\text{N}$ 2D HSQC spectrum of MLDV_vDUF5 in 50 mM Tris-HCl, pH 7.4, containing 500 mM NaCl, acquired at 30°C and 600 MHz (${}^1\text{H}$ frequency).

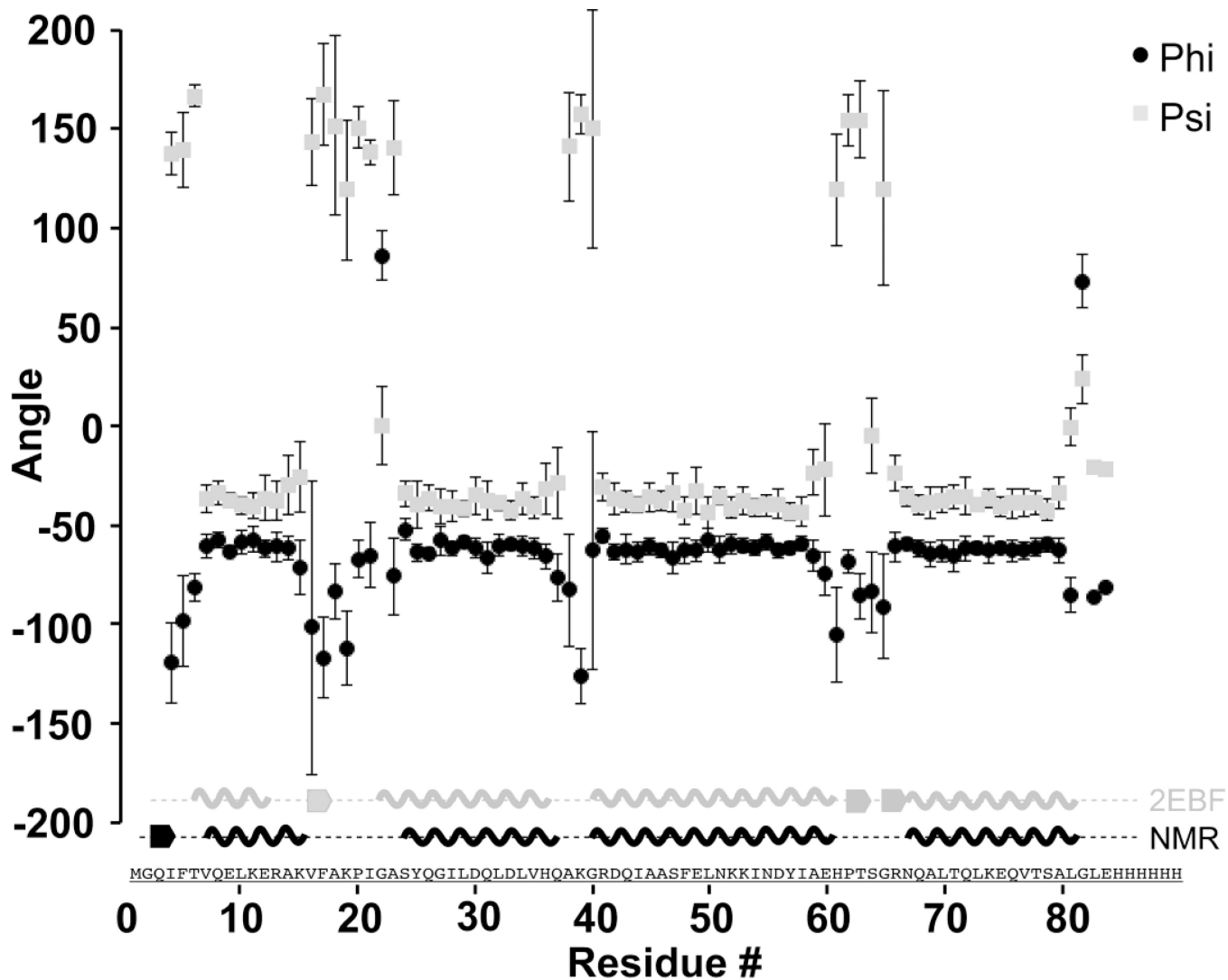


Figure 2.

Secondary structure elements of MLD_vDUF₅ identified by TALOS+ analysis (Shen et al. 2009). Plotted are the Phi (Φ) (black circles) and Psi (Ψ) (gray squares) backbone torsion angles as predicted by TALOS+ analysis (Shen et al. 2009) with error bars. Along the x-axis is a cartoon representation of the secondary structure based on the crystal structure of PMT [PDB 2EBF (grey) (Kitadokoro et al. 2007)] and the secondary structure predicted by TALOS+(black).