Preserved Transmembrane Segment Topology, Structure, and Dynamics in Disparate Micellar Environments

David N. Langelaan¹, Aditya Pandey¹, Muzaddid Sarker¹, and Jan K. Rainey^{1,2,*}

¹Department of Biochemistry & Molecular Biology, Dalhousie University, Halifax NS B3H 4R2, Canada

²Department of Chemistry, Dalhousie University, Halifax NS B3H 4R2, Canada

Abstract

Detergent micelles are frequently employed as membrane mimetics for solution-state membrane protein nuclear magnetic resonance (NMR) spectroscopy. Here, we compare topology, structure, ps-ns timescale dynamics, and hydrodynamics of a model protein with one transmembrane (TM) segment (residues 1-55 of the apelin receptor, APJ, a G-protein-coupled receptor) in three distinct, commonly used micellar environments. In each environment, two solvent-protected helical segments connected by a solvent-exposed kink were observed. The break in helical character at the kink was maintained in a helix-stabilizing fluorinated alcohol environment, implying that this structural feature is inherent. Molecular dynamics simulations also substantiate favorable self-assembly of compact protein-micelle complexes with a more dynamic, solvent exposed kink. Despite the observed similarity in TM segment behavior, micelle-dependent differences were clear in the structure, dynamics, and compactness of the 30-residue, extramembrane N-terminal tail of the protein. This would affect intermolecular interactions and, correspondingly, the functional state of the membrane protein.

Graphical Abstract

*Corresponding author: jan.rainey@dal.ca.

Notes

The authors declare no competing financial interests.

Supporting Information. The supporting information is available free of charge on the ACS Publications website: Experimental methods; Tables S1–S4; Figures S1–S11; and, References in a single PDF.

Solution-state nuclear magnetic resonance (NMR) spectroscopy has become increasingly popular for determination of membrane protein structure,^{1–3} dynamics,^{4–6} and folding.⁷ This relies upon solubilization of a protein with a typically hydrophobic transmembrane (TM) domain flanked by hydrophilic extramembrane regions. Amphipathic membrane mimetics allowing ~isotropic rotational and translational diffusion are thus required. Due to favorable tumbling, ease of supramolecular complex assembly, and availability of membrane mimetic species with a wide variety of hydrophobic and hydrophilic groups, detergent micelles are the most commonly used membrane mimetic.^{1, 3} Excellent agreement between protein structures in micellar vs. bilayer environments has been shown,^{8–12} in one case directly contrasting with the state achieved in multiple crystallization conditions,¹³ although structural agreement between these environments is certainly not always the case.^{3, 14–16} In practice, micelle choice is often based on screening,^{17–18} without predictability as to which micelle will be most amenable to high-resolution NMR.

Micelles are generally spherical or ellipsoidal in shape, with size being influenced by tailgroup length and shape by headgroup properties.¹⁹ Interestingly, there is still debate about micelle size and plasticity, particularly in the context of protein-micelle complexes. Consistent with formation of a detergent annulus around the TM domain of a membrane protein,²⁰ the hydrophobic thickness in a micelle may be considered to be protein-driven versus a bilayer environment where it would be lipid-driven.¹⁶ Despite the apparent malleability of a micelle around a given protein, the plasticity and relatively high local curvature in this environment may perturb both protein structure and function.^{3, 15–16} Competition for hydrogen-bonding between intramolecular donor/acceptor pairs and between solvent or detergent molecules has also been implicated as a potential source of protein conformational sampling.²¹ Such interfacially-driven dynamics may be equally plausible in a bilayer vs. a micellar environment, given similar tailgroup-headgroup and headgroup-solvent interfaces, although a micelle would certainly be more disordered. Beyond these considerations, differential protein dynamics in micellar and bilayer

environments may be particularly pronounced,^{10, 12, 22} leading to potential perturbation of function.

Following the “divide-and-conquer” approach,²³ we recently characterized the extracellular N-terminus and putative first TM segment (TM1) of the apelin receptor (residues 1-55, or AR55), a class A G-protein coupled receptor (GPCR) with wide pathophysiological relevance,²⁴ in dodecylphosphocholine (DPC) micelles using solution-state NMR.²⁵ A solvent-exposed helix kink was identified in TM1, in the vicinity of a hydrophilic and glycine-rich segment with sequence GTTGNG. Although molecular dynamics (MD) simulations of an NMR restraint-informed homology model of full-length AR in solvated phospholipid bilayer showed kink persistence over replicate simulations, it could be argued that this kink is micelle-induced, allowing maximization of favorable solvent exposure of this region of the TM segment. Given other reports of micelle-induced structural perturbation,^{3, 15–16} we felt that AR55 would serve as an excellent model system to test for this phenomenon.

For direct comparison to its state in DPC micelles, AR55 was characterized in two other micelle systems: sodium dodecyl sulfate (SDS), where the tailgroup is identical to DPC but the zwitterionic phosphocholine headgroup is replaced by an anionic sulfate, and lyso-palmitoylphosphatidylglycerol (LPPG), with a more physiological anionic headgroup than SDS and a longer (16- vs. 12-carbon) tailgroup than DPC and SDS. Triple-resonance, spin relaxation, and pulsed-field gradient diffusion NMR data were acquired for each sample of AR55. As an illustration of data quality, ¹H-¹⁵N heteronuclear single quantum coherence (HSQC) spectra of AR55 in SDS, DPC and LPPG micelles exhibit similar peak dispersion and patterns (Figure 1A; assignments annotated in Figures S1–2 in the Supporting Information). Assignment of chemical shifts was achieved for >96% of backbone ¹H, ¹³C, and ¹⁵N and sidechain ¹H chemical shifts, while >67% of sidechain heavy atoms were unambiguously assigned (Table S1).

To compare AR55 solvent exposure in each micelle type, line broadening due to paramagnetic relaxation enhancement upon Mn²⁺ titration was monitored by ¹H-¹⁵N HSQC experiments (Figure S3). Although signal overlap meant that not all positions could be resolved, cross-peaks attributable to residues in the N-terminal tail up to residue L30 as well as residues T43-V49 of AR55 were attenuated by Mn²⁺ in all three types of micelles and, hence, relatively solvent exposed. Other regions of AR55, where assignable, were relatively protected. This indicates that AR55 is not in a canonical micelle-spanning orientation and instead has two micelle-embedded sections with residues T43-V49 being exposed to water regardless of the micellar environment.

Ensembles (retaining lowest energy 40 of 100 calculated structures, Figure 1C–E) were generated in excellent agreement with experimental nuclear Overhauser enhancement (NOE) distance restraints (Figure S4) in SDS and LPPG micelles (previously published DPC ensemble²⁵ shown for comparison) and exhibited good Ramachandran plot statistics (Table S2). Despite NOE restraint coverage over the entire length of the protein (Figure S5), superposition was not possible over the entirety of AR55. Instead, 3–4 regions of highly converged structuring were apparent in each (Figure 1B), as selected²⁶ on the basis of

continuous high ϕ and ψ dihedral angle order parameter (Figures S6–S7) and low root-mean-square deviation (RMSD; Table S3).

Notably, each micelle-embedded section of TM1 formed a converged helix, in agreement with chemical shift index²⁸ and DANGLE²⁹ predictions of secondary structuring alongside canonical³⁰ helical NOE restraint patterns (Figure S4). Relative helical segment orientations varied significantly between ensemble members (reflected in kink angle deviations determined by MC-HELAN,²⁷ Figure 1C–E), consistent with a lack of long-range restraints and decreased constraint at the kink (Figure S5). Despite the differences in micellar environments, the TM1 behavior in SDS and LPPG was entirely consistent with that previously observed²⁵ in DPC.

The solvent-exposed N-terminal tail of AR55 also exhibited converged structuring in the vicinity of the functionally essential³¹ D20 and E23 residues in each micelle (Figure 1C–E). AR55 in SDS and DPC micelles also had independent structurally converged segments N-terminal to this region. Each micellar system, therefore, facilitates AR55 structuring with an anionic face comprising the D20 and E23 sidechains poised²⁵ to bind cationic peptide ligands.

Despite highly similar kinked TM1 behavior in three disparate micelles, this could arise from micellar plasticity and/or isolation of TM1 from the remainder of the GPCR. AR55 behavior was thus examined under the opposite extreme of a hexafluoroisopropanol (HFIP):H₂O (1:1 v:v) mixture. HFIP and other fluorinated alcohols are associated with induction of helical character.^{32–33} These mixtures also favorably solvate both hydrophobic and hydrophilic moieties, sometimes being employed as membrane mimetics.^{23, 34} Based on these properties, we hypothesized that if TM1 can form an uninterrupted helix, it should do so in 50% HFIP.

Following chemical shift (Table S1; Figure S8) and NOE restraint assignment (Table S2; Figures S4–5), greatly facilitated by decreased linewidth and overlap relative to micellar conditions (Figure 1A), a well-converged AR55 structural ensemble was determined in 50% HFIP (Figure 1F). Increased convergence was observed over the TM1 relative to the micellar conditions (Table S3; Figure S9), with retention of a kink at ~G42 in 50% HFIP. Notably, unlike any of the micellar conditions, an extended helical segment consistent with stabilization by HFIP formed in the N-terminal tail of AR55, encompassing residues ~13–27. This extended helical character makes the maintenance of a kink in the TM1 helix in 50% HFIP even more notable.

Polypeptide backbone dynamics, as reflected in ¹⁵N spin relaxation, provide insight both into local motional variation and into global tumbling.³⁵ In each micelle, AR55 ¹⁵N spin relaxation behavior in the micelle-spanning domain including the TM1 kink segregates from the N- and C-terminal tails (Figure 2). Specifically, the tail regions exhibit higher longitudinal relaxation rates (R_1), lower transverse relaxation rates (R_2), and lower ¹H-¹⁵N heteronuclear NOE factors compared to the micelle-embedded TM1 region. This is consistent with the increased variability of tail conformation relative to TM1 exhibited in the structural ensembles. The spin relaxation behavior of AR55 in SDS and DPC micelles is

very similar. In contrast, in LPPG AR55 exhibits lower R_1 in the micelle-embedded region; lower R_2 and heteronuclear NOE in the N-terminal tail; and, higher R_2 in the micelle-embedded region.

^{15}N reduced spectral density mapping³⁶ fits to R_1 , R_2 , and the heteronuclear NOE at 16.4 T were performed³⁷ to estimate $J(0)$, $J(\omega_{\text{N}})$ and $J(0.87\omega_{\text{H}})$ at each resolved backbone amide position for each micelle condition, demonstrating the expected trend³⁸ of $J(0) > J(\omega_{\text{N}}) > J(0.87\omega_{\text{H}})$ (Figure 2). $J(0)$ of the N-terminal tail is largest in DPC micelles and smallest in LPPG micelles. $J(\omega_{\text{N}})$ is very similar throughout AR55 for all of the micelle conditions, with a slight reduction over the micelle-spanning region in LPPG. Finally, $J(0.87\omega_{\text{H}})$ is similar in TM1 for all micelles, but increased in the N-terminal tail in LPPG micelles.

Two factors may be coming into play in the disparate behavior of AR55 in LPPG vs. in DPC and SDS micelles. First, the DPC and SDS detergents were perdeuterated while LPPG was not. R_2 of AR55 should, thus, be elevated through the micelle-spanning region for LPPG by enhanced dipolar cross-relaxation.³⁹ If the surrounding proton versus deuteron bath were the only factor at play, however, R_1 in this region of LPPG would also increase – opposite to the trend observed. Differences in protein-micelle complex tumbling are also a likely contributor to R_1 and R_2 , given the direct dependence of spin relaxation rates upon the summation of the spectral density at various frequencies.⁴⁰

The ratio of R_2/R_1 for backbone amide ^{15}N was used to estimate³⁵ rotational correlation time (τ_{c} ; Table 1) over relatively rigid residues (e.g., with a ^1H - ^{15}N NOE enhancement factor of >0.65 ^{37, 41}), corresponding in AR55 to the micelle-embedded region (Figure 2). Hydrodynamic diameter (d_{H} , from Stokes' law³⁸) was also estimated (Table 1). Diffusion ordered spectroscopy (DOSY)⁴² was employed to characterize translational diffusion (Table S4). As is reasonable for a protein-detergent complex relative to an isolated micelle, a decreased translational diffusion coefficient (D_{C}), with corresponding increased d_{H} (Stokes-Einstein law³⁸), is apparent for each system with AR55 incorporated relative to without (Table 1). Although only estimates assuming spherical behavior, all d_{H} values inferred from DOSY are larger than those from τ_{c} . This difference is most pronounced for the LPPG micelle system, where R_2/R_1 appears to severely underestimate the d_{H} relative to DOSY. This may arise from differences in cross-relaxation contributions between the perdeuterated SDS and DPC relative to protonated LPPG micelles. As a whole, protein-micelle complex translational motion appears more restricted than would be inferred strictly from the ^{15}N spin relaxation behavior of the micelle-embedded portion of the protein.

An unexpected trend is the fact that although AR55 tumbled more slowly and diffused as a much larger protein-detergent complex in LPPG micelles than either DPC or SDS micelles (Table 1), the N-terminal tail in LPPG exhibited increased ps-ns dynamics reflected by decreased $J(0)$ and elevated $J(0.87\omega_{\text{H}})$ (Figure 2). This behavior correlates with the structural ensembles of AR55 (Figure 1C–E), where the N-terminal tail is the least converged in LPPG micelles. One explanation may be an increased compactness of the AR55 N-terminal tail in SDS and DPC micelles, potentially through increased propensity of protein-headgroup interactions relative to LPPG. Increased protection of amide groups in the N-terminal tail from Mn^{2+} -induced PRE was not observed in either DPC or SDS (Figure

S3), implying that any such protein-headgroup interactions are transient in nature. DOSY also demonstrates a greater compactness of the protein-micelle complex in DPC vs. SDS, which may be consistent with a greater propensity for interaction of AR55 with the zwitterionic DPC headgroup over the anionic SDS headgroup.

The solvent exposed kink in TM1 does not exhibit differential dynamics relative to the remainder of the TM segment on the ps-ns time scale probed by ^{15}N nuclear spin relaxation. This implies a single tumbling unit, rather than independent protein-detergent complexes around each helical segment. To better characterize the complexes being formed, molecular dynamics (MD) simulations^{43–45} were employed. AR55-DPC complexes were self-assembled, starting with 54 randomly placed DPC molecules in explicit solvent-filled dodecahedrons containing each of the first four AR55 ensemble members in DPC in four independent simulations. Following self-assembly of stable protein-detergent complexes, on the basis (Figure S10) of converged radius of gyration (R_g) and solvent accessible surface area (SASA), each simulation was allowed to proceed for a further 100 ns. In each trajectory, a single AR55-DPC complex formed and the TM1 kink exhibited lower backbone order and greater variability relative to the remainder of the TM segment (Figure 3). The TM1 kink angle remained relatively stable over the final 100 ns of each trajectory (Figure S11), with distinct kink angle ranges. Each range was also disparate from the kink angle of the NMR ensemble member used as the starting structure for the simulation in question, implying AR55 conformational sampling during the micelle self-assembly period of each simulation with the TM1 conformation becoming relatively fixed once the micelle formed. In the MD simulations, the observed AR55-DPC complex SASA values of $\sim 150 \text{ nm}^2$ (Figure S10B) extrapolate as an upper estimate to spheres of diameter 6.9 nm. This is strikingly similar to the AR55-DPC complex d_H of 6.8 nm inferred by DOSY. Over the MD trajectories, the AR55 N-terminus shows a greater degree of structural plasticity than TM1 but remains in close proximity to the micellar surface (e.g., Figure 3B–C).

In conclusion, despite widely different micelle properties (e.g., d_H in Table 1, headgroups and tailgroups), the TM segment topology and structure of AR55 were consistent across DPC, SDS, and LPPG micelles. MD simulations also recapitulate the NMR-derived structural ensemble, backbone dynamics, and hydrodynamics of AR55 in DPC, with two stable helical segments joined by a solvent exposed and dynamic kink forming a relatively compact protein-micelle complex. Conversely, structure and dynamics of the ~ 30 residue N-terminal tail of AR55 were distinct in each micelle, with a higher degree of tail-micelle interactions implied in DPC and SDS relative to LPPG. This behavior could, in turn, lead to differences in protein functionality, given the potential for variation in conformation and availability for binding as a function of micelle type.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Thanks to Amanda Buyan, Dr. Heidi Koldsø, Dr. Tyler Reddy, Dr. Sara Rouse and Prof. Mark Sansom (Oxford University) for helpful discussions during sabbatical visits and to Bruce Stewart for expert technical support. NMR

experiments were acquired with support from (1) Dr. Ryan McKay at the NANUC facility (Edmonton, AB); (2) Dr. Chris Kirby at Agriculture and Agri-Food Canada (Charlottetown, PE); (3) Dr. Mike Lumsden at the Dalhousie University NMR³ Centre; and, (4) Drs. Ray Syvitki, Nadine Merkley, and Ian Burton at the National Research Council of Canada (NRC) Biomolecular Magnetic Resonance Facility (BMRF, Halifax, NS).

This work was supported by a Canadian Institutes of Health Research (CIHR) Operating Grant (MOP-111138); a Nova Scotia Health Research Foundation Scotia Support Grant (MED-SSG-2015-10041); a Sabbatical Grant from Dalhousie University; and, equipment from the Natural Sciences and Engineering Research Council of Canada (NSERC), the Canadian Foundation for Innovation (Project #15719), and the Dalhousie Medical Research Foundation. The He-cooled probe at the BMRF was provided by Dalhousie University through an Atlantic Canada Opportunities Agency Grant. DNL was the recipient of an NSERC Alexander Graham Bell Doctoral Canada Graduate Scholarship; AP a trainee award from the Beatrice Hunter Cancer Research Institute with funds provided by the Canadian Imperial Bank of Commerce and the Harvey Graham Cancer Research Fund as part of The Terry Fox Strategic Health Research Training Program in Cancer Research at CIHR; and, JKR is supported by a CIHR New Investigator Award.

References

1. Gautier A. Structure Determination of Alpha-Helical Membrane Proteins by Solution-State NMR: Emphasis on Retinal Proteins. *Biochim Biophys Acta*. 2014; 1837:578–588. [PubMed: 23831435]
2. Opella SJ. Structure Determination of Membrane Proteins by Nuclear Magnetic Resonance Spectroscopy. *Annu Rev Anal Chem*. 2013; 6:305–328.
3. Qureshi T, Goto NK. Contemporary Methods in Structure Determination of Membrane Proteins by Solution NMR. *Top Curr Chem*. 2012; 326:123–186. [PubMed: 22160391]
4. Oxenoid K, Chou JJ. A Functional NMR for Membrane Proteins: Dynamics, Ligand Binding, and Allosteric Modulation. *Protein Sci*. 2016; 25:959–973. [PubMed: 26928605]
5. Nishida N, Osawa M, Takeuchi K, Imai S, Stampoulis P, Kofuku Y, Ueda T, Shimada I. Functional Dynamics of Cell Surface Membrane Proteins. *J Magn Reson*. 2014; 241:86–96. [PubMed: 24331735]
6. Reddy T, Rainey JK. Interpretation of Biomolecular NMR Spin Relaxation Parameters. *Biochem Cell Biol*. 2010; 88:131–142. [PubMed: 20453916]
7. Raschle T, Rios Flores P, Opitz C, Muller DJ, Hiller S. Monitoring Backbone Hydrogen-Bond Formation in Beta-Barrel Membrane Protein Folding. *Angew Chem Int Ed Engl*. 2016; 55:5952–5955. [PubMed: 27062600]
8. Franzin CM, Teriete P, Marassi FM. Structural Similarity of a Membrane Protein in Micelles and Membranes. *J Am Chem Soc*. 2007; 129:8078–8079. [PubMed: 17567018]
9. Gautier A, Mott HR, Bostock MJ, Kirkpatrick JP, Nietlispach D. Structure Determination of the Seven-Helix Transmembrane Receptor Sensory Rhodopsin II by Solution NMR Spectroscopy. *Nat Struct Mol Biol*. 2010; 17:768–774. [PubMed: 20512150]
10. Ding Y, Fujimoto LM, Yao Y, Plano GV, Marassi FM. Influence of the Lipid Membrane Environment on Structure and Activity of the Outer Membrane Protein Ail from *Yersinia Pestis*. *Biochim Biophys Acta*. 2015; 1848:712–720. [PubMed: 25433311]
11. Morgado L, Zeth K, Burmann BM, Maier T, Hiller S. Characterization of the Insertase BamA in Three Different Membrane Mimetics by Solution NMR Spectroscopy. *J Biomol NMR*. 2015; 61:333–345. [PubMed: 25638436]
12. Frey L, Lakomek NA, Riek R, Bibow S. Micelles, Bicelles, and Nanodiscs: Comparing the Impact of Membrane Mimetics on Membrane Protein Backbone Dynamics. *Angew Chem Int Ed Engl*. 2017; 56:380–383. [PubMed: 27882643]
13. Separovic F, Killian JA, Cotten M, Busath DD, Cross TA. Modeling the Membrane Environment for Membrane Proteins. *Biophys J*. 2011; 100:2073–2074. author reply 2075. [PubMed: 21504744]
14. Murray DT, Li C, Gao FP, Qin H, Cross TA. Membrane Protein Structural Validation by Oriented Sample Solid-State NMR: Diacylglycerol Kinase. *Biophys J*. 2014; 106:1559–1569. [PubMed: 24739155]
15. Cross TA, Murray DT, Watts A. Helical Membrane Protein Conformations and Their Environment. *Eur Biophys J*. 2013; 42:731–755. [PubMed: 23996195]

16. Zhou HX, Cross TA. Influences of Membrane Mimetic Environments on Membrane Protein Structures. *Annu Rev Biophys.* 2013; 42:361–392. [PubMed: 23451886]
17. Krueger-Koplin RD, Sorgen PL, Krueger-Koplin ST, Rivera-Torres IO, Cahill SM, Hicks DB, Grinius L, Krulwich TA, Girvin ME. An Evaluation of Detergents for NMR Structural Studies of Membrane Proteins. *J Biomol NMR.* 2004; 28:43–57. [PubMed: 14739638]
18. Page RC, Moore JD, Nguyen HB, Sharma M, Chase R, Gao FP, Mobley CK, Sanders CR, Ma L, Sonnichsen FD, et al. Comprehensive Evaluation of Solution Nuclear Magnetic Resonance Spectroscopy Sample Preparation for Helical Integral Membrane Proteins. *J Struct Funct Genomics.* 2006; 7:51–64. [PubMed: 16850177]
19. Oliver RC, Lipfert J, Fox DA, Lo RH, Doniach S, Columbus L. Dependence of Micelle Size and Shape on Detergent Alkyl Chain Length and Head Group. *PLoS One.* 2013; 8:e62488. [PubMed: 23667481]
20. le Maire M, Champeil P, Moller JV. Interaction of Membrane Proteins and Lipids with Solubilizing Detergents. *Biochim Biophys Acta.* 2000; 1508:86–111. [PubMed: 11090820]
21. Page RC, Lee S, Moore JD, Opella SJ, Cross TA. Backbone Structure of a Small Helical Integral Membrane Protein: A Unique Structural Characterization. *Protein Sci.* 2009; 18:134–146. [PubMed: 19177358]
22. Saurel O, Iordanov I, Nars G, Demange P, Le Marchand T, Andreas LB, Pintacuda G, Milon A. Local and Global Dynamics in *Klebsiella Pneumoniae* Outer Membrane Protein a in Lipid Bilayers Probed at Atomic Resolution. *J Am Chem Soc.* 2017; 139:1590–1597. [PubMed: 28059506]
23. Bordag N, Keller S. Alpha-Helical Transmembrane Peptides: A “Divide and Conquer” Approach to Membrane Proteins. *Chem Phys Lipids.* 2010; 163:1–26. [PubMed: 19682979]
24. Chapman NA, Dupre DJ, Rainey JK. The Apelin Receptor: Physiology, Pathology, Cell Signalling, and Ligand Modulation of a Peptide-Activated Class A GPCR. *Biochem Cell Biol.* 2014; 92:431–440. [PubMed: 25275559]
25. Langelaan DN, Reddy T, Banks AW, Dellaire G, Dupre DJ, Rainey JK. Structural Features of the Apelin Receptor N-Terminal Tail and First Transmembrane Segment Implicated in Ligand Binding and Receptor Trafficking. *Biochim Biophys Acta.* 2013; 1828:1471–1483. [PubMed: 23438363]
26. Rainey JK, Fliegel L, Sykes BD. Strategies for Dealing with Conformational Sampling in Structural Calculations of Flexible or Kinked Transmembrane Peptides. *Biochem Cell Biol.* 2006; 84:918–929. [PubMed: 17215879]
27. Langelaan DN, Wiczorek M, Blouin C, Rainey JK. Improved Helix and Kink Characterization in Membrane Proteins Allows Evaluation of Kink Sequence Predictors. *J Chem Inf Model.* 2010; 50:2213–2220. [PubMed: 21090591]
28. 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]
29. Cheung MS, Maguire ML, Stevens TJ, Broadhurst RW. Dangle: A Bayesian Inferential Method for Predicting Protein Backbone Dihedral Angles and Secondary Structure. *J Magn Reson.* 2010; 202:223–233. [PubMed: 20015671]
30. Wüthrich, K. *NMR of Proteins and Nucleic Acids.* Wiley; New York: 1986.
31. Zhou N, Zhang X, Fan X, Argyris E, Fang J, Acheampong E, DuBois GC, Pomerantz RJ. The N-Terminal Domain of APJ, a CNS-Based Coreceptor for HIV-1, Is Essential for Its Receptor Function and Coreceptor Activity. *Virology.* 2003; 317:84–94. [PubMed: 14675627]
32. Sonnichsen FD, Van Eyk JE, Hodges RS, Sykes BD. Effect of Trifluoroethanol on Protein Secondary Structure: An NMR and CD Study Using a Synthetic Actin Peptide. *Biochemistry.* 1992; 31:8790–8798. [PubMed: 1390666]
33. Hirota N, Mizuno K, Goto Y. Cooperative Alpha-Helix Formation of Beta-Lactoglobulin and Melittin Induced by Hexafluoroisopropanol. *Protein Sci.* 1997; 6:416–421. [PubMed: 9041644]
34. Buck M. Trifluoroethanol and Colleagues: Cosolvents Come of Age. *Recent Studies with Peptides and Proteins. Q Rev Biophys.* 1998; 31:297–355. [PubMed: 10384688]

35. Kay LE, Torchia DA, Bax A. Backbone Dynamics of Proteins as Studied by ^{15}N Inverse Detected Heteronuclear NMR Spectroscopy: Application to Staphylococcal Nuclease. *Biochemistry*. 1989; 28:8972–8979. [PubMed: 2690953]
36. Farrow NA, Zhang O, Szabo A, Torchia DA, Kay LE. Spectral Density Function Mapping Using ^{15}N Relaxation Data Exclusively. *J Biomol NMR*. 1995; 6:153–162. [PubMed: 8589604]
37. Spyropoulos L. A Suite of Mathematica Notebooks for the Analysis of Protein Main Chain ^{15}N NMR Relaxation Data. *J Biomol NMR*. 2006; 36:215–224. [PubMed: 17061025]
38. Cavanagh, J., Fairbrother, W., Palmer, AG., III, Rance, M., Skelton, NJ. *Protein NMR Spectroscopy: Principles and Practice*. 2. Academic Press; New York: 2007.
39. Markus MA, Dayie KT, Matsudaira P, Wagner G. Effect of Deuteration on the Amide Proton Relaxation Rates in Proteins. Heteronuclear NMR Experiments on Villin 14T. *J Magn Reson B*. 1994; 105:192–195. [PubMed: 7952934]
40. Abragam, A. *The Principles of Nuclear Magnetism*. Clarendon Press; Oxford, UK: 1961.
41. Tjandra N, Feller SE, Pastor RW, Bax A. Rotational Diffusion Anisotropy of Human Ubiquitin from ^{15}N Nmr Relaxation. *J Am Chem Soc*. 1995; 117:12562–12566.
42. Morris KF, Johnson CS. Diffusion-Ordered 2-Dimensional Nuclear Magnetic Resonance Spectroscopy. *J Am Chem Soc*. 1992; 114:3139–3141.
43. Berendsen HJC, Vandespoel D, Vandrunen R. GROMACS: A Message-Passing Parallel Molecular Dynamics Implementation. *Computer Physics Communications*. 1995; 91:43–56.
44. Hess B, Kutzner C, van der Spoel D, Lindahl E. GROMACS 4: Algorithms for Highly Efficient, Load-Balanced, and Scalable Molecular Simulation. *J Chem Theory Comput*. 2008; 4:435–447. [PubMed: 26620784]
45. Schmid N, Eichenberger AP, Choutko A, Riniker S, Winger M, Mark AE, van Gunsteren WF. Definition and Testing of the GROMOS Force-Field Versions 54A7 and 54B7. *Eur Biophys J*. 2011; 40:843–856. [PubMed: 21533652]

Figure 1.

(A) ^1H - ^{15}N HSQC spectra at 16.7 T of AR55 solubilized in indicated conditions (note: dashed box aliased). (B) Schematic illustration NMR structural ensembles with grey segments sampling conformations relative to superposed black segment. (C–F) NMR structural ensembles of AR55 in SDS- d_{25} (C), DPC- d_{38} (D²⁵), LPPG (E), or 50% HFIP (F); backbone of representative structure shown, with all ensemble members superposed over converged regions;²⁶ ensemble average ($\pm$ average deviation) kink angles²⁷ between the two AR TM1 helical segments shown.

Figure 2. R_1 , R_2 , ^1H - ^{15}N heteronuclear NOE enhancement factors, and corresponding reduced spectral density mapping³⁶ fits at given frequency ($J(\omega)$) as a function of AR55 residue in indicated micelle determined at 16.4 T. Shaded areas represent regions protected from water.

Figure 3.

(A) ϕ and ψ dihedral angle order parameter (S)⁴⁵ over 100 ns MD simulations following self-assembly of an AR55-DPC micelle with each of the four lowest energy NMR ensemble members (grey shading represents regions protected from water according to PRE). (B–C) Frames from trajectory #1 at 30 ns (B) and 100 ns (C), following micelle self-assembly (DPC headgroups cyan, tailgroups tan, AR55 colored as in Figure 1).

Table 1

Comparative rotational (τ_c and d_H ; estimated³⁵ from R_2/R_1 of AR55) and translational (d_H ; estimated by DOSY⁴²) diffusion behavior of given detergent-AR55 complexes, alongside d_H of micelles in absence of AR55.

Detergent	τ_c (ns) (AR55 in complex)	d_H (nm)		
		Rotational (AR55)	Translational (complex)	Translational (micelle)
DPC	10.0 ns	5.2	6.8	4.9
SDS	12.3 ns	4.9	7.3	4.9
LPPG	17.0 ns	5.8	9.4	8.5