Supporting Information for:

Impact of Bilayer Lipid Composition on the Structure and Topology of the Transmembrane Amyloid Precursor C99 Protein

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Contents

Supporting Material and Methods

Chemicals. The following chemicals and abbreviations were used, as detailed below: bovine milk sphingomyelin (MSM; Avanti Polar Lipids, Alabaster Alabama), chicken egg sphingomyelin (ESM; Avanti Polar Lipids), cholesterol (Ch, Sigma, St. Louis, Missouri), 1,2-dihexanoyl-*sn*-glycero-3-phosphocholine (DHPC; Avanti Polar Lipids), 3-[(3-cholamidopropyl)dimethylammonio]-2-hydroxy-1-propanesulfonate (CHAPSO, Affymetrix-Anatrace), 3-[(3-cholamidopropyl)dimethylammonio]-1-propanesulfonate **(**CHAPS), 16-doxylstearic acid (16-DSA; Sigma), 1,2-dimyristoyl-*sn*-glycero-3-phosphocholine (DMPC; Avanti Polar Lipids), 1,2-dipalmitoyl-*sn*-glycero-3-phosphocholine (DPPC; Genzyme, Cambridge, Massachusetts), Gd(III)-diethylenetriaminepentaacetic acid (Gd-DTPA; Sigma), lyso-myristoylphosphatidylglycerol (LMPG, Avanti Polar Lipids), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phosphocholine (POPC; Avanti Polar Lipids), 1-palmitoyl-2-oleoyl-*sn*-glycero-3-phospho-(1'-rac-glycerol) (POPG; Avanti Polar Lipids); porcine brain sphingomyelin (BSM; Avanti Polar Lipids), Deuterium oxide (D_2O, Sigma-Aldrich).

Bicelle Screening. DMPC, DPPC, POPC, POPG, MSM, BSM and ESM were weighed out as solids and mixed in glass vials at the molar ratios indicated in Table S1. The lipid mixtures were solubilized with 95:5 benzene:ethanol to homogenize the components and then freeze-dried overnight to yield white powders. Aqueous detergent stock solutions in 50 mM PIPES buffer pH 7.0 of βOG, DHPC, CHAPS, or CHAPSO, were added to the lipid powders to reach the indicated molar q ratio where q = moles lipid/moles detergent, with the final lipid+detergent w/v percentage being set to 10% (w/v). Bicelle mixtures were allowed to equilibrate at room temperature for one hour. Solubility was recorded. Freeze-thaw rounds were then performed

on samples showing incomplete solubility. The glass vials were frozen in liquid nitrogen and then thawed in room temperature water. After five rounds of freeze-thawing, solubility was recorded. Incompletely soluble samples were then treated to bath sonication for up to one hour. Incompletely soluble samples were sometimes subjected to additional freeze-thaw cycles, with thawing in the presence of bath sonication at 55-60°C. As a last resort, samples that remained insoluble were heated briefly in boiling water followed by vortexing.

Dynamic Light Scattering. Dynamic light scattering measurements were conducted at room temperature (24 °C) using the DynaPro instrument (Protein Solutions, Inc. Charlottesville, VA) and data were analyzed by *Dynamics V5* software (Protein Solutions, Inc.). Selected bicelles were prepared in water at 4% total bicelle concentration (lipid plus detergent) in addition to the critical micelle concentration of free DHPC. For each sample three independent experiments were performed, with at least 40 light scattering scans on 100 µL samples per experiment. The 100% mass peak was chosen for analysis. *Dynamics V5* software was used to calculate molecular translational diffusion coefficients, D_T, by fitting the data to an exponential autocorrelation function. The program then used the Stokes-Einstein equation for an isotropic globular assembly to calculate the hydrodynamic radius, R_h: $D_T = kT/6\pi\eta R_h$, where k is the Boltzmann constant, T is the experimental temperature, and η is the solvent viscosity*. It is recognized that the bicelle samples of this work are almost certainly not spherical aggregates, so we emphasize that the calculated R_h and aggregate molecular weights are only apparent—useful for qualitatively comparing bicelle to bicelle, but not quantitatively accurate.*

Bicelles for Protein Purification. DMPC, POPC, MSM, and ESM were weighed in solid form and mixed in glass vials at the desired molar ratio. The lipid mixtures were then solubilized with 95:5 benzene:ethanol and freeze-dried overnight to yield a white powder. After the solvent was completely removed, stock solutions of 10% DHPC in water were added to reach the indicated molar q 0.5. Bicelle solutions were brought to final volume with water and concentrated imidazole buffer to reach final conditions with a bicelle concentration of 2%, 250 mM imidazole and pH 7.8.

Protein Expression and Purification. The C-terminal domain of the human amyloid precursor protein (C99) was expressed essentially as described previously.[1,2] In brief, cDNA encoding human C99, tagged with a hexa-histidine purification tag –QGRILQISITLAAALEHHHHHH at its C-terminus, was integrated into a modified pET-21a vector. The protein was expressed in BL21DE3 E coli cells in ^{15}N-labeled M9 media. Three grams of cells were lysed in 60 ml lysis buffer (20 mM Tris, 300 mM NaCl, pH 7.8) with lysozyme solution (0.2 mg/ml) and sonication (5 s on and 5 s off at a power of 40 W), followed by centrifugation to isolate the inclusion bodies, with 3 times washing in the lysis buffer. The inclusion bodies were then solubilized in 30 ml SDS-urea solution (8M urea, 20 mM Tris, 150 mM NaCl, pH 7.8, and 0.2% SDS). The solution was subjected to centrifugation at 15000 x g to remove insoluble debris and the supernatant, containing SDS/urea-solubilized C99, was incubated with 5 ml of the SDS-urea solution pre-equilibrated with Ni-NTA-Superflow metal ion affinity chromatography resin (Qiagen, Valencia, CA) at room temperature for 90 min. The resin was extensively washed with 200 mL of Tris-buffered saline (20 mM Tris, 150 mM NaCl, pH 7.8) containing 0.2% SDS and then washed with 7.5 mL of a 1% bicelle (w/v) solution (containing 20 mM imidazole and pH 7.8) in a gravity column at room temperature. C99 was eluted with 10 mL of a 2% bicelle buffer containing 250 mM imidazole at pH 7.8. A chart recorder coupled with a UV detector was used to monitor the elution at 280 nm. Typically, the C99 was eluted in 5 mL. The typical concentration of purified C99 in 5 mL of the 2% bicelle elution solution was 20–30 µM (calculated with A_{280} and ε_{280} = 5960 M^{-1} cm^{-1}).

Preparation of NMR Samples. The ca. 5 ml C99 solution in 2% bicelles was concentrated to 0.5 ml with an Amicon Ultra centrifugal filter cartridge (molecular weight cut-off = 10 kDa). The pH of the concentrated sample was then adjusted to 4.5 with acetic acid. D_2O and EDTA were then added to 10% and 1 mM, respectively, before running NMR experiments. The concentrated C99 sample (200 µl) was transferred into a 3 mm NMR tube for the paramagnet-free control NMR spectrum acquisition. After the control spectrum acquisition, an aliquot of a stock solution of 200 mM Gd(III)-DTPA in water at pH 4.5 was added directly to the NMR tube (200 µL C99 NMR sample) to a final concentration of 0.8 mM. The spectrum with Gd-DTPA was then acquired using identical NMR acquisition parameters as the diamagnetic control. When 16-DSA was the paramagnetic probe, a different method was used to add this compound to the control sample. A stock solution of 16-DSA in methanol (74 µl) with a concentration of 2.5 mg/ml (6.5 mM) was dispensed into a 1 ml glass vial and then dried in a fume hood overnight. The concentrated C99 NMR solution (200 µl) was added to the glass vial. The sample became clear after vigorously vortexing. The final concentration of 16-DSA in the NMR sample was then 2.4 mM. This sample was then subjected to NMR spectral acquisition using the same parameters as used for the diamagnetic control sample. All NMR experiments were conducted at 45°C on a 900 MHz NMR spectrometer using the standard Bruker ^{1}H,^{15}N-TROSY pulse sequence. The TROSY spectra from LMPG and the bicelle mixtures are shown in Figures 1 and S1.

Preparation of Reduced Salt NMR Samples. C99 protein was purified as above, but was eluted from the gravity NTA column with an elution buffer containing 2% bicelle, 500 mM imidazole. The sample was concentrated to 0.5 mL using an Amicon Ultra Centrifugal filter with a MWCO of 10 kDa. The concentrated C99 sample (0.5 mL) in high salt concentration (500 mM imidazole) was then diluted with 4.5 mL 15 mM (the CMC of DHPC) DHPC solution and concentrated back to 0.5 mL. The imidazole concentration was reduced to 5 mM by repeating the buffer exchange step twice. D_2O and EDTA were added to the sample to 10% and 1 mM, respectively. Acetate buffer was added to 35 mM and pH was adjusted to 4.5. The sample was then transferred to an NMR tube as described above. The LMPG samples was prepared slightly differently in that C99 was eluted from the NTA column using a solution containing 0.5% LMPG instead of 2% bicelles. Also, this sample was buffer exchanged only once and no sodium acetate was added. The final imidazole concentration in the LMPG sample was 50 mM.

Chemical Shift Analysis. Amide ^{1}H and ^{15}N chemical shifts were measured for C99 in each bicelle mixture. ^{1}H,^{15}N-TROSY peak assignments for C99 spectra in the novel types of bicelles examined in this work were completed based on correlating peaks to the previously-assigned peaks in DMPC-DHPC bicelles.[1] Backbone amide chemical shifts in both ^{1}H and ^{15}N dimensions for each residue in different bicelle conditions were compared to the reference chemical shift for that residue in a random coil structure, with the sequence-dependent correction based on its preceding and following residues (Figures 2 and S3).[3] Specifically, the reference chemical shift of the residue i in a random coil structure is determined by averaging the statistical value for the residue i (from http://www.bmrb.wisc.edu/published/Ikura_cs_study/part2_rc_aa_cs_stats.pdf), with the neighboring effect from the residue i-1 (from http://www.bmrb.wisc.edu/published/Ikura_cs_study/part2_dipep_NH-.pdf for ^{1}H and http://www.bmrb.wisc.edu/published/Ikura_cs_study/part2_dipep_N-.pdf for ^{15}N), and the statistical value of the residue i corrected with the neighboring effect from the residue i+1 (from http://www.bmrb.wisc.edu/published/Ikura_cs_study/part2_dipep_NH+.pdf for 1H and http://www.bmrb.wisc.edu/published/Ikura_cs_study/part2_dipep_N+.pdf for ^{15}N). All statistical values used in this study were accessed in Oct 2013. The chemical shift differences were

plotted as a function of the residue number using Origin 8.0 (OriginLab Corp, Northampton, MA).

Examination of the Topology of C99 in Bicelles. In each bicelle condition, the NMR spectrum with Gd-DPTA or 16-DSA was overlaid with the control NMR spectrum (see examples in Figure S7). The intensity ratio for each assigned peak in the presence of either Gd-DPTA or 16-DSA relative to its corresponding peak in the control spectrum was determined using Sparky (T. D. Goddard and D. G. Kneller, SPARKY 3, University of California, San Francisco). The intensity ratio of each peak was plotted (Figure 3) as a function of its residue number using Origin 8.0 (OriginLab Corp, Northampton, MA).

Table S1. Potential bicelle compositions tested for this work. *Mixtures for which it was possible to achieve complete solubilization of all components are indicated in bold font.*

Lipids	Detergent	q ratio	Observations
4:1 POPC:POPG + 15mol% Ch	**DHPC**	**0.5**	**Quickly solubilized upon adding detergent solution and a few rounds of freeze-thaw; final solution clear at room temperature.**
1:1 Ch:ESM	DHPC	0.5	Did not solubilize upon adding detergent solution, freeze-thaw, or hot-sonication; further, did not solubilize upon reducing q to q = 0.3 or q = 0.15. Final solution thick and densely cloudy.
1:1:1 Ch:ESM:DMPC	DHPC	0.5	Did not solubilize upon adding detergent solution, freeze-thaw, or hot-sonication; further, did not solubilize upon reducing q to q = 0.3 or q = 0.15. Final solution thick and densely cloudy.
6:2:2 Ch:ESM:POPC	DHPC	0.5	Did not solubilize upon adding detergent solution, freeze-thaw, or hot-sonication; further, did not solubilize upon reducing q to q = 0.3 or q = 0.15. Final solution thick and densely cloudy.
4:1 POPC:POPG + 15mol% Ch	**CHAPS**	**0.5**	**Quickly solubilized upon adding detergent solution and a few rounds of freeze-thaw; final solution clear at room temperature.**
1:1 Ch:ESM	CHAPS	0.5	Did not solubilize upon adding detergent solution, freeze-thaw, or hot-sonication; further, did not solubilize upon reducing q to q = 0.3 or q = 0.15. Final solution thick and densely cloudy.
1:1:1 Ch:ESM:DMPC	CHAPS	0.5	Did not solubilize upon adding detergent solution, freeze-thaw, or hot-sonication; further, did not solubilize upon reducing q to q = 0.3 or q = 0.15. Final solution thick and densely cloudy.
6:2:2 Ch:ESM:POPC	CHAPSO	0.5	Did not solubilize upon adding detergent solution, freeze-thaw, or hot-sonication; further, did not solubilize upon reducing q to q = 0.3 or q = 0.15. Final solution thick and densely cloudy.
70:15:15 POPC:Ch:ESM	DHPC	0.5	Somewhat clarified upon adding detergent, upon several freeze thaws and hot sonication, left with clear solution containing several white particulates; reduction to q = 0.2 did not improve solubility.
85:15 ESM:Ch	DHPC	0.5	Somewhat clarified upon several freeze thaws and hot sonication but still slightly cloudy; reduction to q = 0.2 did not improve solubility.
10:4 POPC:ESM	**DHPC**	**0.5**	**Clarified completely upon detergent addition and vortexing.**
9:1 ESM:Ch	DHPC	0.5	Slightly clarified upon detergent addition; after continuous hot sonication for one hour, solution became mostly clear but some particulates remained.
9:1 BSM:Ch	DHPC	0.5	Slightly clarified upon detergent addition; after continuous hot sonication for one hour, solution became mostly clear but some particulates remained.
10:4:1 POPC:ESM:Ch	**DHPC**	**0.5**	**Slightly clarified upon detergent addition; after continuous hot sonication for one hour, solution became completely clear; solution remained clear at room temperature.**
6:3:1 DMPC:ESM:Ch	DHPC	0.5	At first clarified during freeze thaw but returned to cloudy at room temperature
3:3:1 DMPC:ESM:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
8:1 ESM:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
3:1 ESM: Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
1.7:1 ESM:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
1:1 POPC:BSM	**DHPC**	**0.5**	**Solubilized slowly upon addition of detergent.**

Lipids	Detergent	q ratio	Observations
2:1 POPC:BSM	**DHPC**	**0.5**	**Solubilized slowly upon addition of detergent and short sonication.**
1:1 POPC:MSM	**DHPC**	**0.5**	**Milky solution after addition of detergent. Improved to clear after cycles of hot sonication and freeze-thaw.**
2:1 POPC:MSM	**DHPC**	**0.5**	**Milky solution after addition of detergent. Improved to clear after cycles of hot sonication and freeze-thaw.**
2:1 BSM:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve after sonicating, freeze/thaw, or boiling.
2:1 MSM:Ch	DHPC		Milky solution after addition of detergent. Did not improve after sonicating, freeze/thaw, or boiling.
ESM	**DHPC**	**0.5**	**Solubilized slowly upon addition of detergent**
MSM	**DHPC**	**0.5**	**Solubilized slowly upon addition of detergent and short sonication.**
BSM	**DHPC**	**0.5**	**Solubilized slowly upon addition of detergent**
6:3:1 DMPC:ESM:Ch	CHAPSO	0.5	Clear solution with white solids present. Did not improve with hot sonication, freeze/thaw, or boiling.
3:3:1 DMPC:ESM:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw or hot sonication. Slight improvement with boiling, although still cloudy.
8:1 ESM:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
3:1 ESM: Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
1.7:1 ESM:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
1:1 POPC:BSM	**CHAPSO**	**0.5**	**Solubilized slowly upon addition of detergent and short sonication.**
2:1 POPC:BSM	**CHAPSO**	**0.5**	**Solubilized slowly upon addition of detergent and short sonication.**
1:1 POPC:MSM	**CHAPSO**	**0.5**	**Slowly solubilized to clear solution upon addition of detergent.**
2:1 POPC:MSM	**CHAPSO**	**0.5**	**Slowly solubilized to clear solution upon addition of detergent.**
2:1 BSM:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
2:1 MSM:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
MSM	**CHAPSO**	**0.5**	**Dissolved slowly to clear on benchtop.**
4:1 DPPC:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
2:1 DPPC:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
1:1:1 DPPC:ESM:Ch	DHPC	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
4:1 DPPC:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
2:1 DPPC:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.
1:1:1 DPPC:ESM:Ch	CHAPSO	0.5	Milky solution after addition of detergent. Did not improve with freeze-thaw, hot-sonication, or boiling.

Table S2. Results of dynamic light scattering (DLS) measurements for selected 4% (w/v) bicelle mixtures in water at 24 °C. R_h values were calculated from a globular (spherical) model and thus are only approximations of a discoidal bicelle shape. *It is recognized that the bicelle samples of this work are almost certainly not spherical aggregates, so we emphasize that the calculated R_h and aggregate molecular weights are only apparent—useful for qualitatively comparing the relative sizes from bicelle to bicelle, but not quantitatively accurate.*

Bicelle Composition	D_T (10^{-9} cm^2/s)	R_h (nm)	MW (kDa)	PolyD (nm)	% PolyD	PolyD Index
DMPC-DHPC q = 0.5	1016±1	2.44 ±0.01	27.2±0.1	0.336±0.001	13.7±0.1	0.020±0.01
POPC-DHPC q = 0.5	952±3	2.57±0.01	30.6±0.1	0.351±0.002	13.6±0.1	0.020±0.01
2:1 POPC:MSM-DHPC q = 0.5	955±3	2.57±0.03	30.5±0.7	0.353±0.004	13.7±0.1	0.020±0.01
MSM-DHPC q = 0.5	972±3	2.53±0.01	29.6±0.3	0.356±0.001	14.0±0.1	0.020±0.01
ESM-DHPC q = 0.5	962±3	2.58±0.01	31.0±0.2	0.358±0.001	13.9±0.1	0.020±0.01

Supporting References

(1) Barrett, P. J.; Song, Y.; Van Horn, W. D.; Hustedt, E. J.; Schafer, J. M.; Hadziselimovic, A.; Beel, A. J.; Sanders, C. R. *Science* **2012**, *336*, 1168.

(2) Beel, A. J.; Mobley, C. K.; Kim, H. J.; Tian, F.; Hadziselimovic, A.; Jap, B.; Prestegard, J. H.; Sanders, C. R. *Biochemistry* **2008**, *47*, 9428.

(3) Schwarzinger, S.; Kroon, G. J.; Foss, T. R.; Chung, J.; Wright, P. E.; Dyson, H. J. *Journal of the American Chemical Society* **2001**, *123*, 2970.

Supporting Figures and Captions

Figure S1. 900 MHz ^{1}H-^{15}N TROSY spectra of C99 in LMPG and in the 5 different bicelle compositions used in this work. All samples contained 0.2-0.3 mM C99, 20% w/v bicelles, 250 mM imidazole, 1 mM EDTA, 10% D2O and pH 4.5. In all cases the bicelle q ratio (lipid-to-detergent mol/mol) was 0.5. The temperature was 45 °C. The data for LMPG micelles were previously reported[1] and represents pH 6.5 conditions.

Figure S1

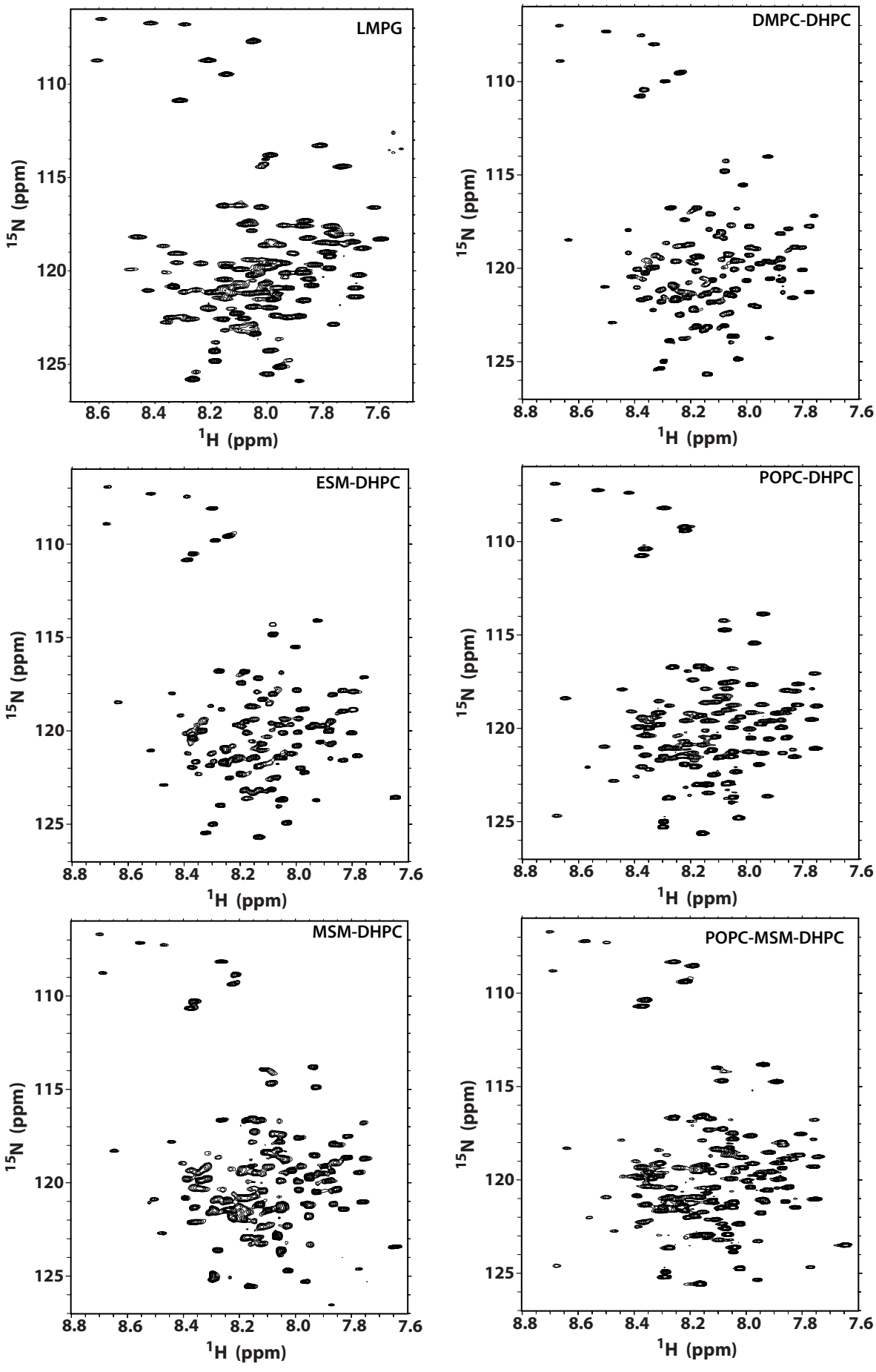

LMPG
DMPC-DHPC
ESM-DHPC
POPC-DHPC
MSM-DHPC
POPC-MSM-DHPC
^{15}N (ppm)
^{1}H (ppm)
110
115
120
125
8.8
8.6
8.4
8.2
8.0
7.8
7.6

Figure S2. Backbone amide ^{15}N chemical shift differences between C99 in LMPG micelles versus C99 in various bicelles in both high salt (green bars) and low salt (red bars) conditions. POPC-MSM-DHPC bicelle experiments were not performed in low salt conditions. The vertical lines demarcate boundaries of C99 domains defined according to the C99 structure determined in LMPG micelles.[1] The residues marked with cyan bars are either invisible or lack peak assignments. The y-axis scale is the same as in Figure 2.

Figure S2

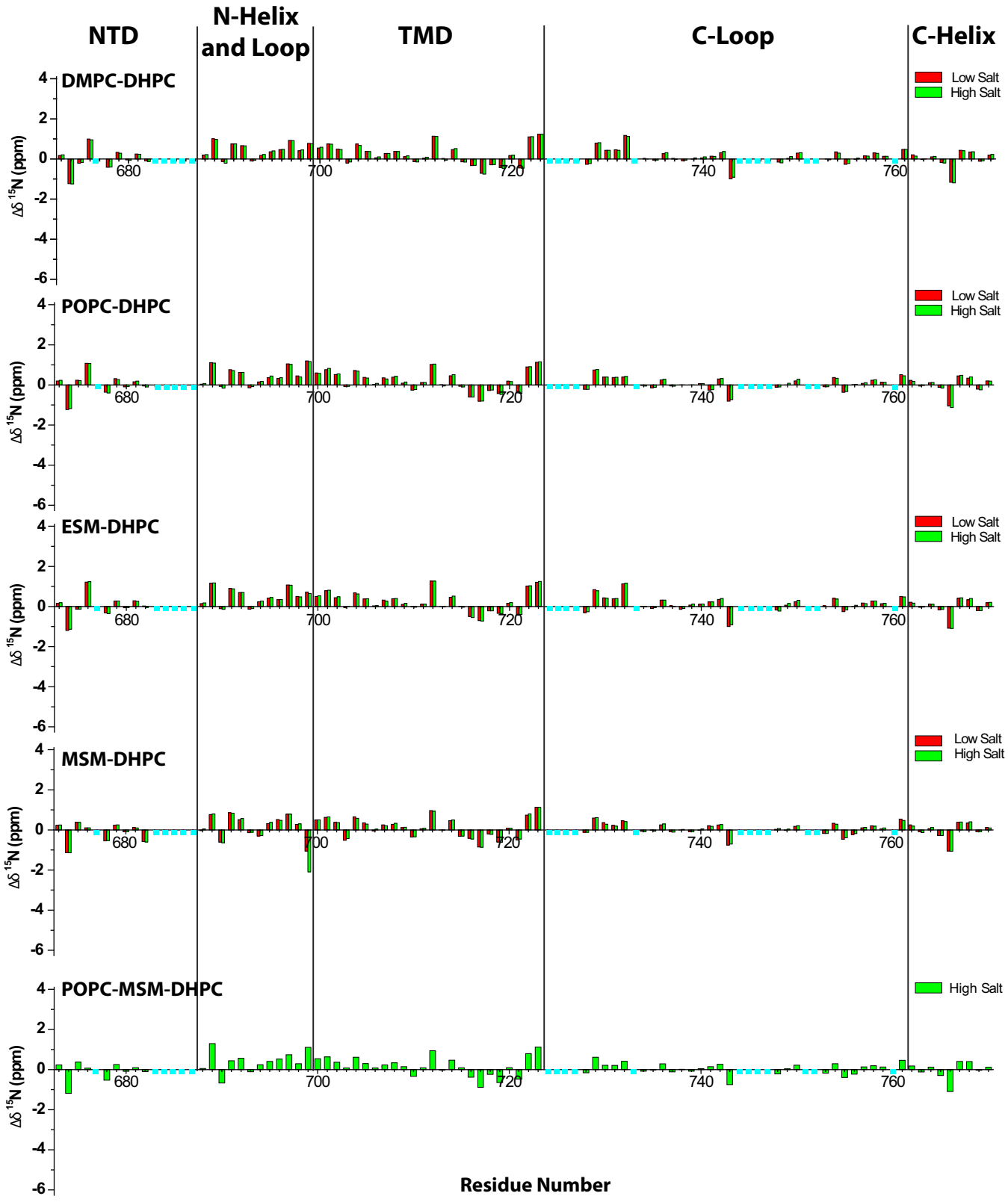

NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
DMPC-DHPC
POPC-DHPC
ESM-DHPC
MSM-DHPC
POPC-MSM-DHPC
Low Salt
High Salt
Δδ ¹⁵N (ppm)
Residue Number

Figure S3. Residue-specific backbone amide ^{1}H chemical shifts for C99 in various bicelles. The values reported here represent the difference between the reported chemical shift and the random coil chemical shift. The residues marked with cyan bars are invisible (even in the absence of a paramagnet) or lack peak assignments The four vertical lines represent the boundaries of the N terminal cytosolic domain (NTD, 672-687), the combined N-helix and N-loop (688-699), the transmembrane domain (TMD, 700-723), the C-loop (724-761), and the distal C-terminal domain (C-helix, 762-770).[1] All samples contained 0.2-0.3 mM C99, 20% w/v bicelles, 250 mM imidazole, 1 mM EDTA, 10% D2O and pH 4.5. In all cases the bicelle q ratio (lipid-to-detergent mol-to-mol ratio) was 0.5. The temperature was 45 °C. The data for LMPG micelles were previously reported in Beel et al., 2008[2] and represents pH 6.5 conditions.

Figure S3

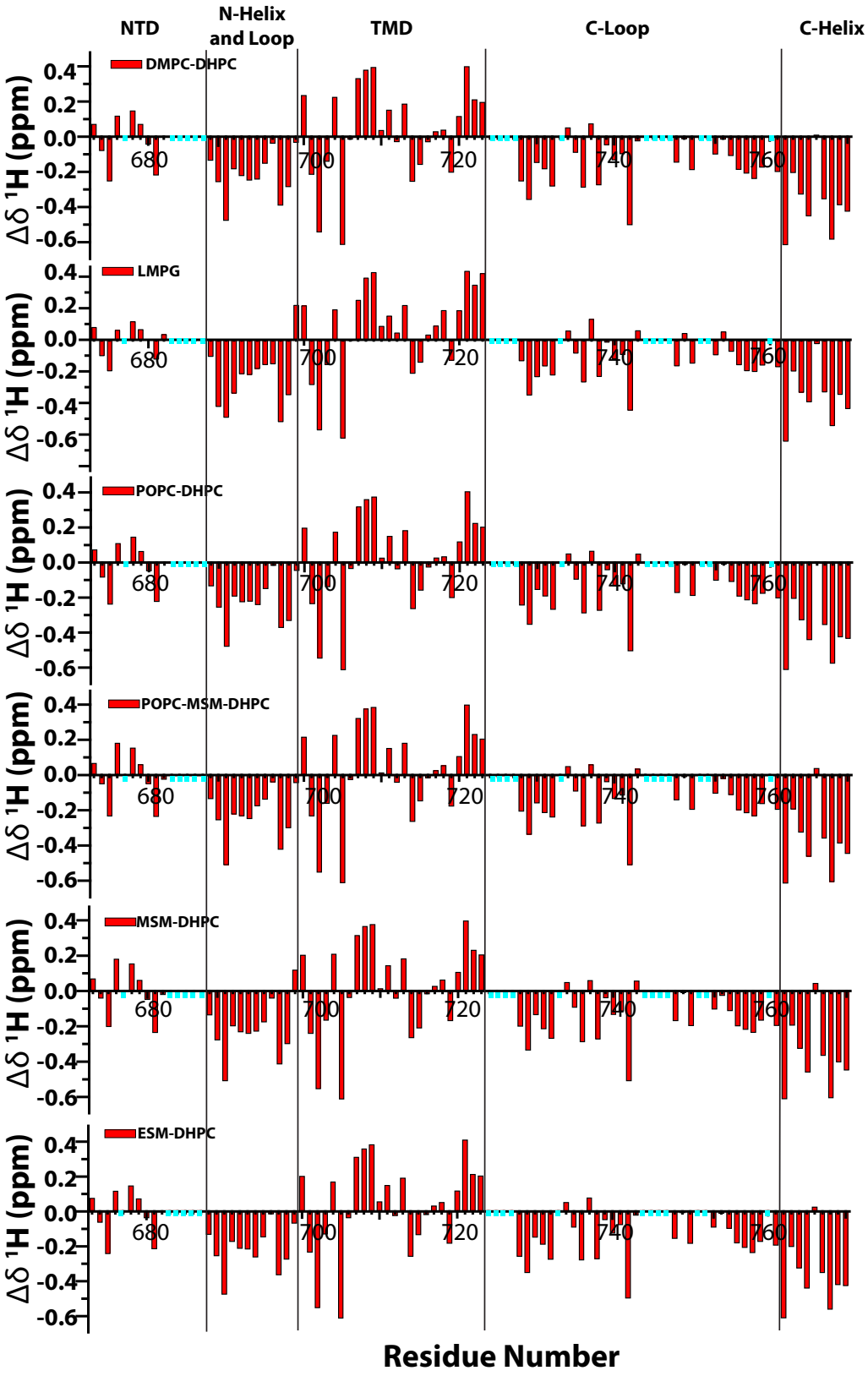

NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
DMPC-DHPC
LMPG
POPC-DHPC
POPC-MSM-DHPC
MSM-DHPC
ESM-DHPC
Δδ ¹H (ppm)
0.4
0.2
0.0
-0.2
-0.4
-0.6
680
700
720
740
760
Residue Number

Figure S4. Backbone amide ^{1}H chemical shift differences between C99 in LMPG micelles versus C99 in various bicelles in both high salt (green bars) and low salt (red bars) conditions. POPC-MSM-DHPC bicelle experiments were not performed in low salt conditions. POPC-MSM-DHPC bicelle experiments were not performed in low salt conditions. The vertical lines demarcate boundaries of C99 domains defined according to C99 structure determined in LMPG micelles.[1] The residues marked with cyan bars are either invisible or lack peak assignments. The y-axis scale is the same as in Figure S3.

Figure S4

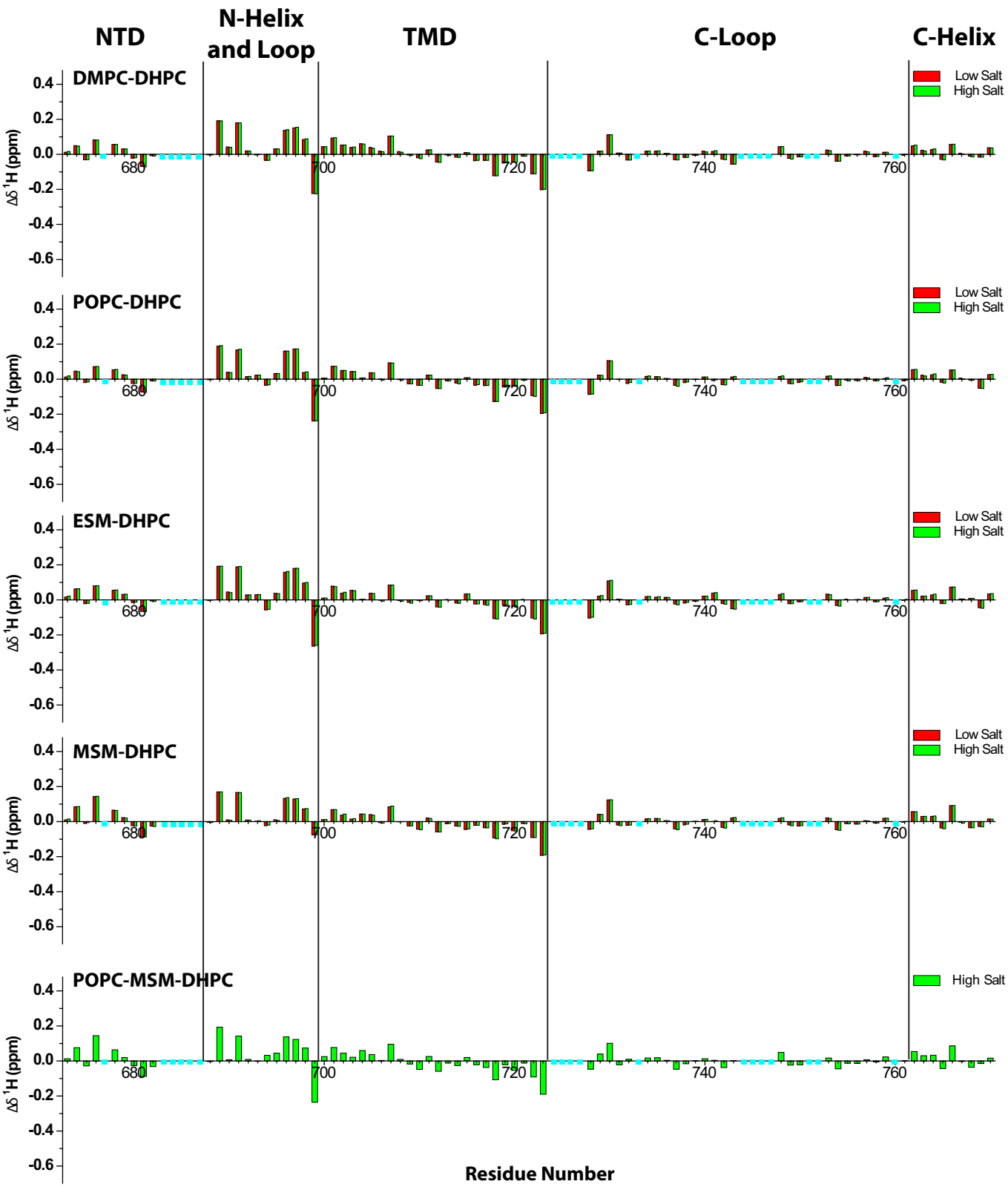

NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
DMPC-DHPC
POPC-DHPC
ESM-DHPC
MSM-DHPC
POPC-MSM-DHPC
Low Salt
High Salt
Δδ ¹H (ppm)
0.4
0.2
0.0
-0.2
-0.4
-0.6
680
700
720
740
760
Residue Number

Figure S5. Comparison of residue-specific backbone amide ^{15}N chemical shifts for C99 in LMPG micelles and in various DHPC-based bicelles under reduced salt conditions (red bars) versus high salt (green bars) conditions. The high salt data is the same that is presented in Figure 2 of the paper. The values reported here represent the difference between the measured chemical shift and the random coil chemical shift (estimated as described in the Supporting Methods section). The residues marked with cyan bars are either too broad to observe (even in the absence of a para-magnet) or lack peak assignments. The four vertical lines represent the boundaries of the disordered N terminal cytosolic domain (NTD, 672-687), the combined N-helix and N-loop (688-699), the transmembrane domain (TMD, 700-723), the C-loop (724-761), and the distal C-terminal domain (C-helix, 762-770).[1] Comparison of residue-specific backbone amide 15N chemical shifts for C99 in LMPG micelles and in various DHPC-based bicelles under reduced salt conditions (red bars) versus high salt (green bars) conditions. The high salt data is the same that is presented in Figure 2 of the paper. All reduced salt samples except for the LMPG sample contained 0.2-0.3 mM C99, 20% w/v bicelles (q =0.5), 5 mM imidazole, 35 mM sodium acetate 1 mM EDTA, 10% D2O and pH 4.5. The reduced salt LMPG sample was identical except that it contained 50 mM imidazole, no sodium acetate, and was pH 6.5. The temperature was 45 °C.

Figure S5

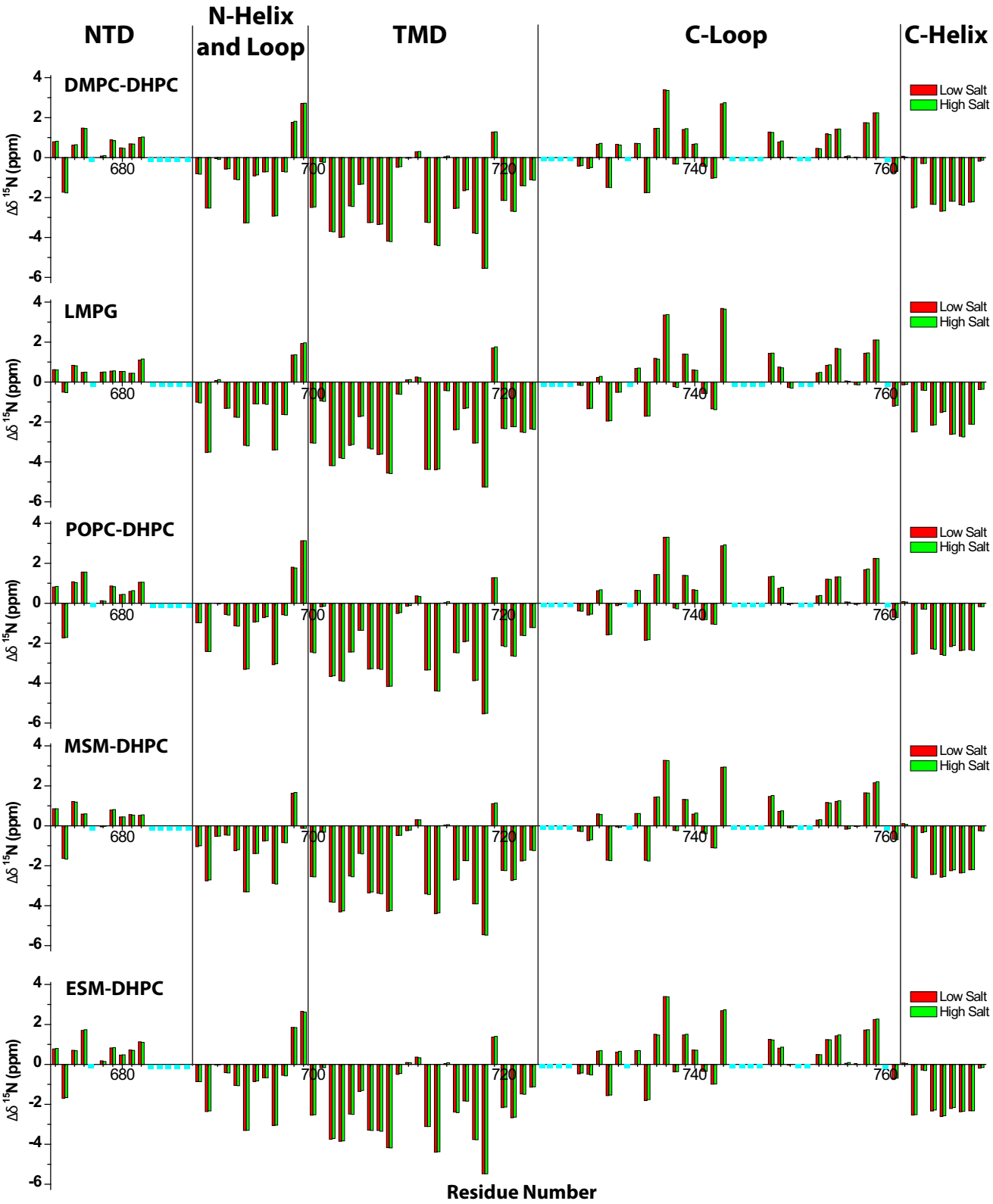

NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
DMPC-DHPC
LMPG
POPC-DHPC
MSM-DHPC
ESM-DHPC
Low Salt
High Salt
Δδ ¹⁵N (ppm)
Residue Number

Figure S6. Comparison of residue-specific backbone amide ^{1}H chemical shifts for C99 in LMPG micelles and in various DHPC-based bicelles under reduced salt conditions (red bars) versus high salt (green bars) conditions. The high salt data is the same that is presented in Figure 2 of the paper. The values reported here represent the difference between the reported chemical shift and the random coil chemical shift (estimated as described in the Supporting Methods section). The residues marked with cyan bars are invisible (even in the absence of a paramagnet) or lack peak assignments. The four vertical lines represent the boundaries of the N terminal cytosolic domain (NTD, 672-687), the combined N-helix and N-loop (688-699), the transmembrane domain (TMD, 700-723), the C-loop (724-761), and the distal C-terminal domain (C-helix, 762-770).[1] The composition of the high salt samples is given in the caption to Figure 2 of the paper. All reduced salt samples except for the LPMG sample contained 0.2-0.3 mM C99, 20% w/v bicelles, 5 mM imidazole, 35 mM sodium acetate, 1 mM EDTA, 10% D2O and pH 4.5. The reduced salt LMPG sample was identical that except it contained 50 mM imidazole, no sodium acetate, and was pH 6.5. In all cases the bicelle q ratio (lipid-to-detergent mol-to-mol ratio) was 0.5. The temperature was 45 °C.

Figure S6

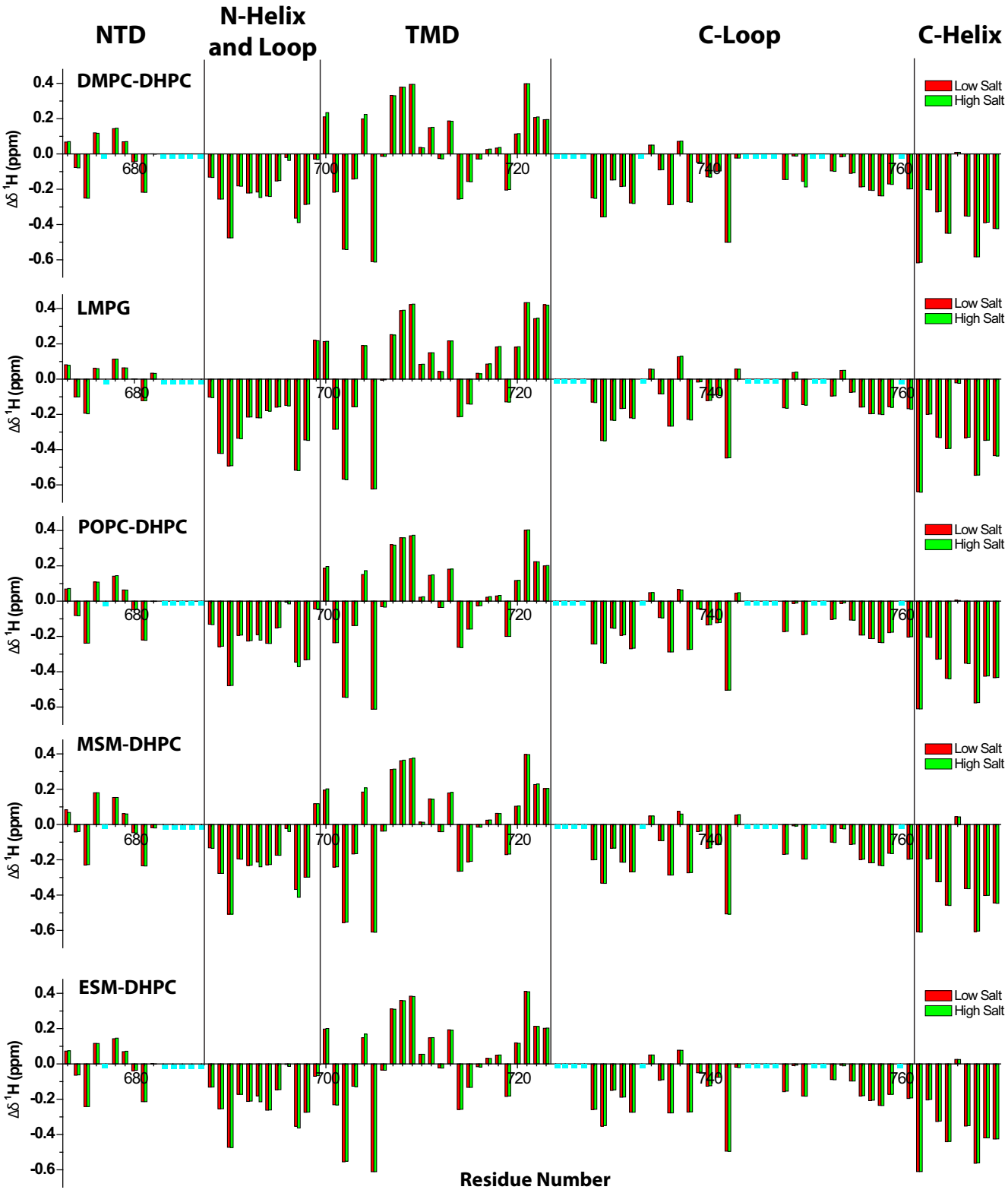

NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
DMPC-DHPC
LMPG
POPC-DHPC
MSM-DHPC
ESM-DHPC
Low Salt
High Salt
Δδ ¹H (ppm)
0.4
0.2
0.0
-0.2
-0.4
-0.6
680
700
720
740
760
Residue Number

Figure S7. Example of data used to measure accessibility of C99 backbone amide sites in bicelles to the water soluble paramagnetic chelate Gd(III)-DTPA (left panel) or to the lipophilic nitroxide spin-labeled 16-doxylstearate (16-DSA, right panel). The black spectrum is a diamagnetic reference spectrum, while the red spectrum is from a matched sample except that it also contains the paramagnetic probe. NMR acquisition parameters were also matched for each pair of spectra. These 900 MHz $^{1}H,^{15}N$-TROSY spectra were acquired at 45°C and are for q = 0.5 DHPC-ESM bicelles.

Figure S7

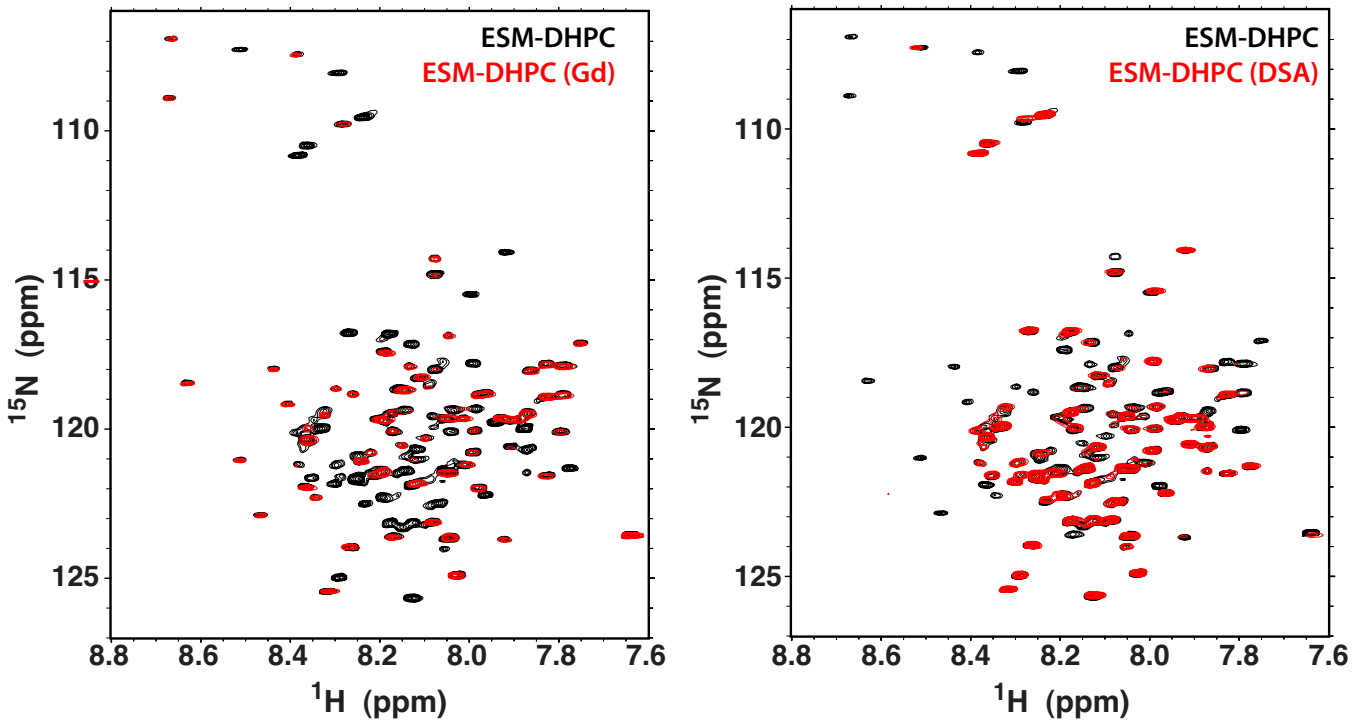
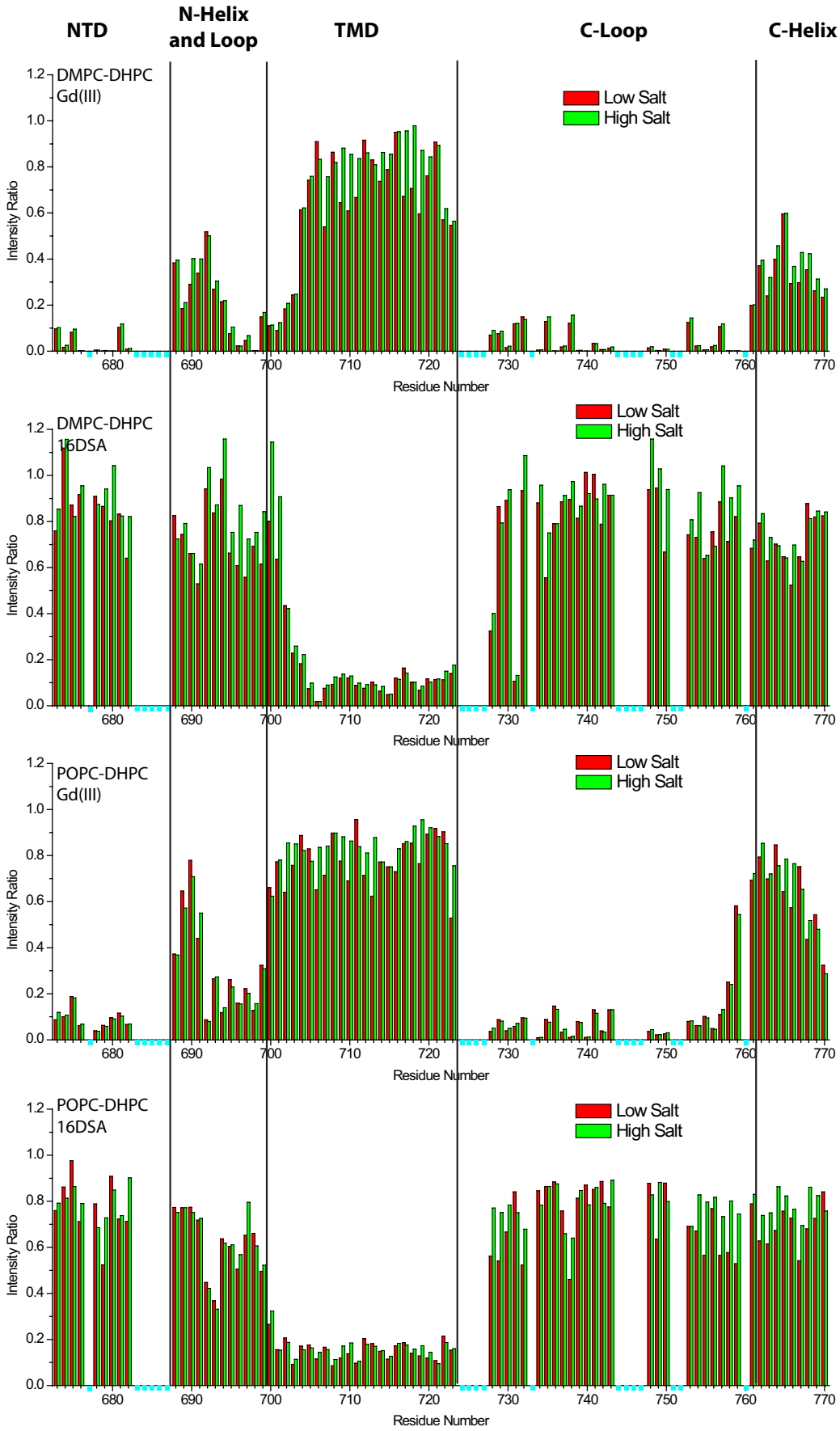
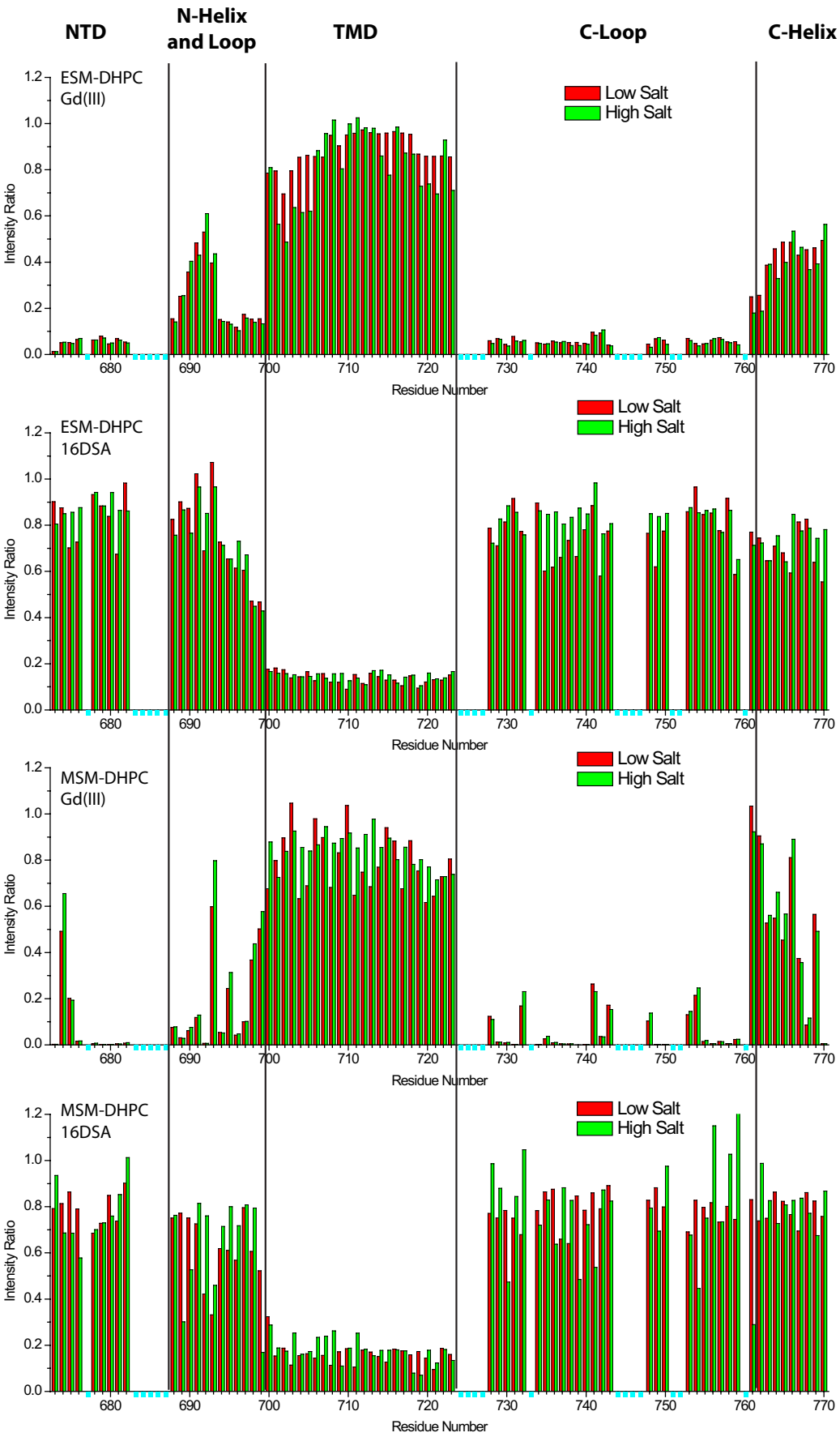
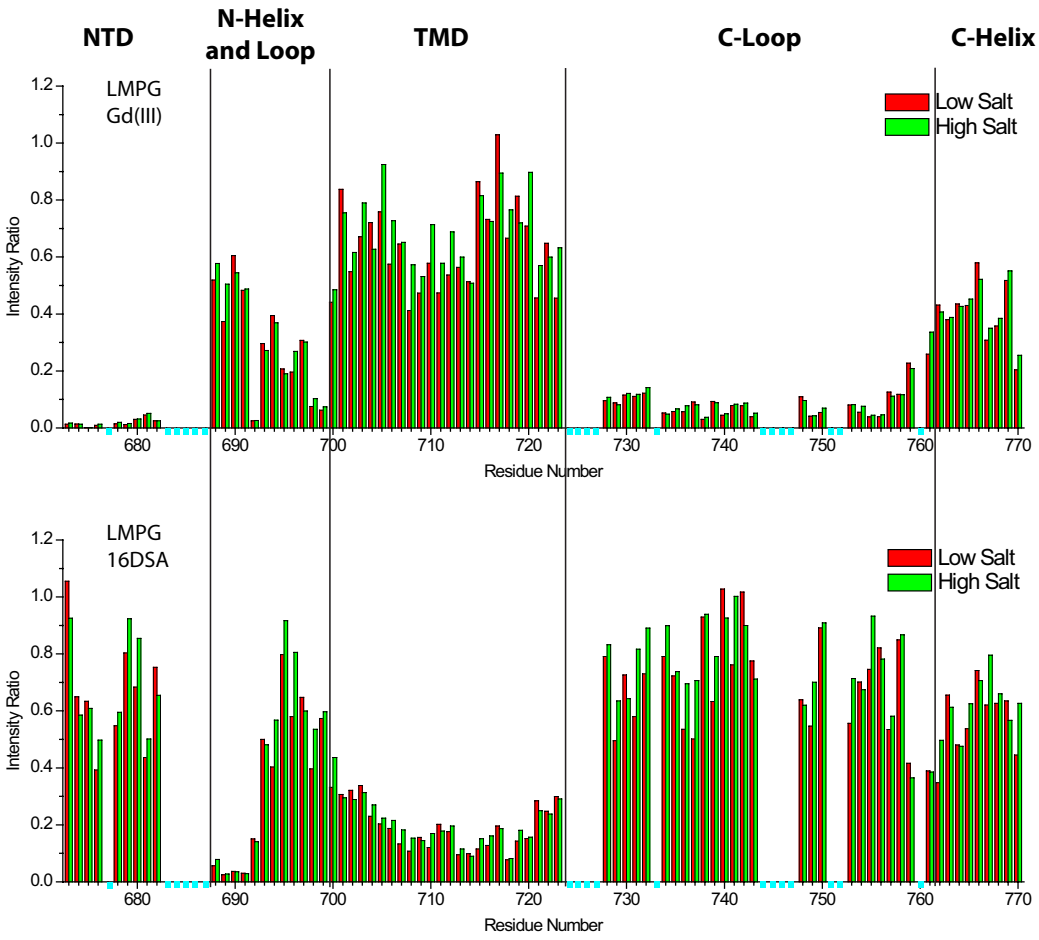

ESM-DHPC
ESM-DHPC (Gd)
ESM-DHPC
ESM-DHPC (DSA)
^{15}N (ppm)
^{1}H (ppm)
110
115
120
125
8.8
8.6
8.4
8.2
8.0
7.8
7.6

Figure S8 (3 pages). Comparison of paramagnetic probe access NMR data for C99 in bicelles and LMPG micelles under reduced salt (35-50 mM) versus high salt (250 mM) conditions. The high salt data is the same that is presented in Figure 3 of the paper and is represented as green bars in these plots. The low salt data (red bars) is for samples of composition as given in Figs. S5 and S6 plus the added 16-DSA and Gd(III)-DTPA paramagnetic probes (at the same concentrations as given in the caption to Figure 3 of the paper). We did not collect low salt data for the POPC-MSM-DHPC bicelles. These data document both the general insensitivity of the C99 topology to salt concentration and also the generally high reproducibility of the probe access data.


NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
DMPC-DHPC Gd(III)
DMPC-DHPC 16DSA
POPC-DHPC Gd(III)
POPC-DHPC 16DSA
Low Salt
High Salt
Intensity Ratio
Residue Number
0.0
0.2
0.4
0.6
0.8
1.0
1.2
680
690
700
710
720
730
740
750
760
770


NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
ESM-DHPC Gd(III)
ESM-DHPC 16DSA
MSM-DHPC Gd(III)
MSM-DHPC 16DSA
Low Salt
High Salt
Intensity Ratio
Residue Number
0.0
0.2
0.4
0.6
0.8
1.0
1.2
680
690
700
710
720
730
740
750
760
770


NTD
N-Helix and Loop
TMD
C-Loop
C-Helix
LMPG
Gd(III)
Low Salt
High Salt
Intensity Ratio
Residue Number
LMPG
16DSA
Low Salt
High Salt
Intensity Ratio
Residue Number