*Table of Contents:*

**Figure S1:** A) $^{15}$N-HSQC NMR spectrum of ($^{15}$N-$^{13}$C)-labeled gp36-MPER in DPC/SDS 90:10 M/M mixed micelles; B) folded $^{13}$C-HSQC NMR spectrum of ($^{15}$N-$^{13}$C)-labeled gp36-MPER in DPC/SDS 90:10 M/M mixed micelles.

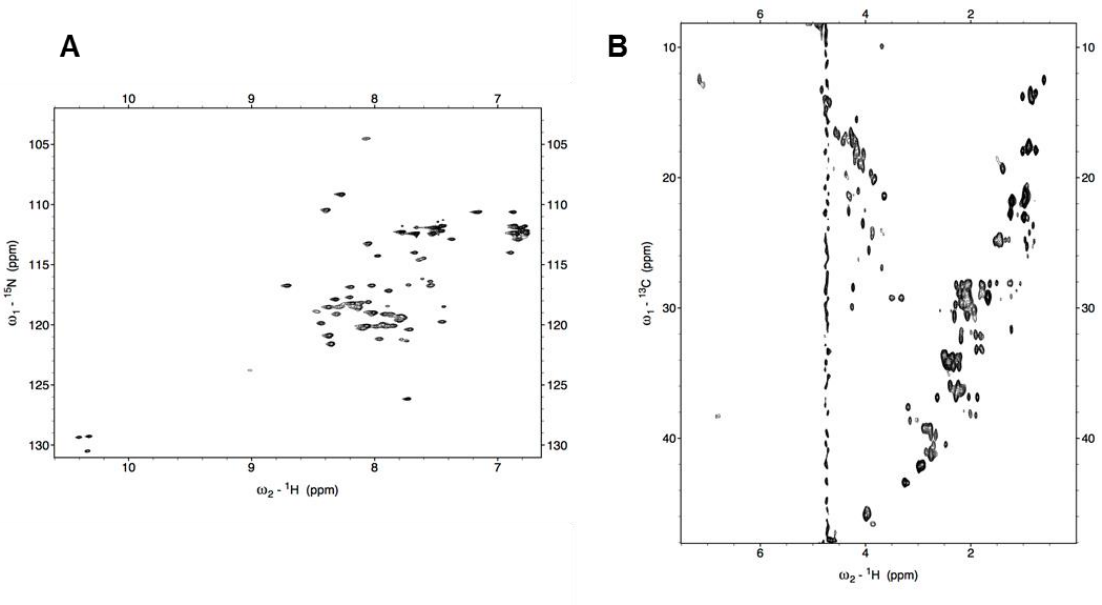

**Table S1:** TALOS+ prediction backbone dihedral angles of 0.5 mg ($^{15}N$-$^{13}C$)-labeled gp36 CHR-MPER acquired on Bruker 900 MHz in DPC/SDS 90:10 M/M mixed micelles**,** at pH 7.4 and at 300 K. Gp36 CHR-MPER backbone dihedral angles (PHI, PSI), estimated standard deviations of the prediction errors in PHI and PSI (DPHI, DPSI), TALOS+ database matching score (DIST), Wishart RCI chemical shift order parameter ($S^2$), number of database triplets used to form the torsion angle predictions (COUNT, CS COUNT), classification of the prediction result (CLASS; **None** if no torsion prediction was made, **Good** if majority consensus in database matches, **Warn** if no consensus in database matches, **Dyn** if RCI $S^2$ value indicates dynamic conformation).

| RESID | RESNAME | PHI (°) | PSI (°) | DPHI (°) | DPSI (°) | DIST | $S^2$ | COUNT | CS_COUNT | CLASS |
|---|---|---|---|---|---|---|---|---|---|---|
| **740** | T | -59.27 | -32.743 | 6.798 | 6.694 | 0.295 | 0.82 | 25 | 13 | **Strong** |
| **741** | K | -64.099 | -36.692 | 3.451 | 5.224 | 0.198 | 0.838 | 25 | 15 | **Strong** |
| **742** | D | -69.767 | -38.96 | 3.261 | 4.211 | 0.139 | 0.864 | 25 | 14 | **Strong** |
| **743** | L | -64.344 | -42.725 | 3.785 | 3.874 | 0.119 | 0.872 | 25 | 14 | **Strong** |
| **744** | Q | -61.889 | -42.434 | 2.942 | 3.434 | 0.118 | 0.88 | 25 | 14 | **Strong** |
| **745** | Q | -64.923 | -41.272 | 1.866 | 2.816 | 0.123 | 0.875 | 25 | 14 | **Strong** |
| **746** | K | -65.752 | -40.177 | 3.738 | 4.614 | 0.136 | 0.875 | 25 | 14 | **Strong** |
| **747** | F | -67.009 | -36.821 | 4.576 | 5.526 | 0.181 | 0.88 | 25 | 14 | **Strong** |
| **748** | Y | -64.819 | -33.798 | 3.675 | 6.293 | 0.265 | 0.865 | 25 | 14 | **Strong** |
| **749** | E | -66.576 | -34.207 | 6.072 | 6.355 | 0.479 | 0.837 | 25 | 13 | **Strong** |
| **750** | I | -71.134 | -33.281 | 8.714 | 9.908 | 0.685 | 0.823 | 10 | 12 | **Generous** |
| **751** | I | -70.167 | -33.55 | 12.121 | 10.156 | 0.628 | 0.839 | 25 | 12 | **Strong** |
| **752** | L | -64.437 | -41.439 | 3.934 | 4.164 | 0.499 | 0.867 | 25 | 13 | **Strong** |
| **753** | D | -66.624 | -39.424 | 4.273 | 3.586 | 0.333 | 0.88 | 25 | 13 | **Strong** |
| **754** | I | -64.585 | -41.763 | 3.305 | 3.019 | 0.218 | 0.87 | 25 | 14 | **Strong** |
| **755** | E | -64.433 | -37.712 | 3.857 | 7.155 | 0.188 | 0.85 | 25 | 14 | **Strong** |
| **756** | Q | -66.81 | -29.89 | 4.93 | 7.798 | 0.195 | 0.79 | 25 | 15 | **Strong** |
| **757** | N | -72.646 | -23.666 | 8.89 | 9.49 | 0.249 | 0.675 | 25 | 15 | **Strong** |
| **758** | **N** | -83.381 | -9.776 | 13.122 | 14.316 | 0.301 | 0.578 | 10 | 15 | **Dyn** |
| **759** | **V** | -85.832 | -17.811 | 11.078 | 12.641 | 0.347 | 0.549 | 7 | 15 | **Dyn** |
| **760** | **Q** | -59.901 | 127.866 | 5.762 | 5.666 | 0.377 | 0.574 | 25 | 13 | **Dyn** |
| **761** | **G** | 80.971 | 4.017 | 10.068 | 14.434 | 0.67 | 0.596 | 25 | 13 | **Dyn** |
| **762** | **K** | -119.08 | 32.449 | 13.865 | 27.416 | 1.519 | 0.584 | 5 | 13 | **Dyn** |
| **763** | **T** | -81.612 | 138.095 | 27.123 | 13.574 | -1.319 | 0.59 | 25 | 13 | **Dyn** |
| **764** | **G** | 87.54 | 4.72 | 9.69 | 15.78 | 1.065 | 0.652 | 25 | 12 | **Strong** |
| **765** | I | -83.032 | -23.771 | 21.661 | 19.913 | 0.665 | 0.732 | 6 | 12 | **Warn** |
| **766** | Q | -64.897 | -37.011 | 6.741 | 7.086 | 0.508 | 0.797 | 25 | 14 | **Strong** |
| **767** | Q | -65.743 | -40.861 | 4.731 | 5.737 | 0.497 | 0.798 | 25 | 14 | **Strong** |
| **768** | L | -64.078 | -40.361 | 4.498 | 4.57 | 0.558 | 0.792 | 25 | 13 | **Strong** |
| **769** | Q | -67.642 | -35.127 | 4.154 | 4.969 | 0.479 | 0.761 | 25 | 13 | **Strong** |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| **770** | K | -67.202 | -36.082 | 5.542 | 5.681 | 0.429 | 0.777 | 25 | 13 | **Strong** |
| **771** | W | -70.467 | -33.251 | 11.365 | 13.761 | 0.383 | 0.809 | 25 | 14 | **Strong** |
| **772** | E | -63.52 | -41.008 | 5.068 | 6.5 | 0.318 | 0.869 | 25 | 14 | **Strong** |
| **773** | D | -67.762 | -37.224 | 4.255 | 5.09 | 0.298 | 0.884 | 25 | 15 | **Strong** |
| **774** | W | -64.26 | -43.256 | 3.666 | 6.78 | 0.43 | 0.868 | 25 | 14 | **Strong** |
| **775** | V | -65.844 | -35.363 | 7.173 | 7.915 | 0.559 | 0.853 | 25 | 11 | **Strong** |
| **776** | G | -65.321 | -35.277 | 4.082 | 7.93 | 0.755 | 0.826 | 8 | 10 | **Warn** |
| **777** | W | -65.683 | -36.749 | 5.126 | 8.095 | 0.782 | 0.808 | 25 | 10 | **Strong** |
| **778** | **I** | -79.538 | -15.175 | 13.499 | 16.625 | 0.862 | 0.756 | 25 | 11 | **Strong** |
| **779** | **G** | -72.287 | -19.263 | 11.195 | 6.852 | 0.938 | 0.708 | 4 | 12 | **Warn** |
| **780** | **N** | -95.479 | -8.29 | 13.461 | 12.633 | 0.686 | 0.64 | 10 | 12 | **Generous** |
| **781** | **I** | -70.999 | 136.664 | 6.686 | 11.093 | 0.799 | 0.627 | 25 | 9 | **Strong** |
| **782** | **P** | -58.878 | 143.966 | 6.645 | 8.142 | 0.811 | 0.662 | 25 | 8 | **Strong** |
| **783** | Q | -59.959 | -34.931 | 3.728 | 5.313 | 0.996 | 0.72 | 25 | 8 | **Strong** |
| **784** | Y | -74.76 | -18.594 | 15.94 | 20.997 | 0.948 | 0.727 | 25 | 12 | **Strong** |
| **785** | L | -65.858 | -30.25 | 8.291 | 7.025 | 0.817 | 0.715 | 25 | 8 | **Strong** |

**Table S2:** $^{1}H$, $^{15}N$ and $^{13}C$ chemical shift of 0.5 mg ($^{15}N$-$^{13}C$)-labeled gp36-MPER acquired on Bruker 900 MHz in DPC/SDS 90:10 M/M mixed micelles**.** The final pH was 7.4. NMR experiments were recorded at 300 K.

| Number Resudue | Residue | HN | $^{15}N$ | $^{13}C\alpha$ | H$\alpha$ | $^{13}C\beta$ | H$\beta$ | H$\gamma$ | Others |
|---|---|---|---|---|---|---|---|---|---|
| **738** | **Q** | | | 54.736 | 4.752 | 30.450 | 2.615/2.311 | | |
| **739** | **T** | 8.752 | 117.073 | 65.468 | 4.290 | 68.366 | 4.155 | | |
| **740** | **K** | 8.387 | 120.983 | 58.262 | 4.217 | 32.055 | 1.851 | | |
| **741** | **D** | 7.796 | 119.367 | 56.514 | 4.610 | 40.956 | 2.821 | | |
| **742** | **L** | 7.741 | 120.066 | 58.351 | 4.020 | | 1.677 | | QQδ 0.924 |
| **743** | **Q** | 8.337 | 117.844 | 60.124 | 3.833 | 28.693 | 2.258 | | |
| **744** | **Q** | 7.932 | 117.201 | 58.798 | 4.134 | 28.051 | 2.249 | | |
| **745** | **K** | 7.929 | 118.588 | 58.332 | 4.177 | | 1.983 | 1.559 | Qδ 1.804 |
| **746** | **F** | 8.368 | 111.615 | 61.053 | 4.203 | 38.576 | 3.135 | | Qδ 7.111 |
| **747** | **Y** | 8.128 | 117.472 | 61.007 | 4.122 | 37.938 | 3.189 | | Qε 7.115<br>Cε1 92.892 |
| **748** | **E** | 8.088 | 117.921 | 58.059 | 4.010 | | 2.081 | 2.337 | |
| **749** | **I** | 7.918 | 119.996 | 59.189 | 4.063 | | 1.896 | 1.524 | Qδ1 0.793 |
| **750** | **I** | 7.831 | 118.343 | 60.388 | 3.825 | | 1.919 | 1.266/1.011 | Qδ1 0.830 |
| **751** | **L** | 7.938 | 120.068 | 62.522 | 3.999 | | 1.701 | | QQδ 0.807 |
| **752** | **D** | 7.733 | 119.459 | 56.789 | 4.514 | 41.197 | 2.711 | | |
| **753** | **I** | 8.003 | 120.007 | 64.227 | 3.876 | | 1.906 | 1.080/0.894 | |
| **754** | **E** | 8.389 | 121.040 | 58.836 | 4.068 | 29.435 | 2.124 | | |
| **755** | **Q** | 8.221 | 117.395 | 57.850 | 4.133 | 28.789 | 2.152 | | |
| **756** | **N** | 8.127 | 116.880 | 54.230 | 4.663 | 39.125 | 2.807 | | |
| **757** | **N** | 8.238 | 118.284 | 53.950 | 4.748 | 39.115 | 2.897/2.730 | | |
| **758** | **V** | 8.047 | 119.072 | 63.518 | 4.042 | 32.100 | 2.195 | 0.955 | |
| **759** | **Q** | 8.405 | 121.028 | 56.755 | 4.255 | 28.856 | 2.104/2.017 | | |
| **760** | **G** | 8.247 | 108.979 | 45.809 | 4.121 | | | | |
| **761** | **K** | 8.136 | 120.069 | 57.020 | 4.188 | 33.020 | 1.830 | 1.335 | |
| **762** | **T** | 8.101 | 113.101 | 61.134 | 4.338 | 69.847 | 4.650 | | |
| **763** | **G** | 8.437 | 110.298 | 45.914 | 3.963 | | | | |
| **764** | **I** | 8.165 | 120.090 | 59.331 | 4.026 | | 1.909 | 1.420/0.894 | |
| **765** | **Q** | 8.420 | 121.495 | 58.691 | 4.098 | 28.690 | 2.350/2.062 | | |
| **766** | **Q** | 8.154 | 118.402 | 57.098 | 4.239 | 28.641 | 2.058 | | |
| **767** | **L** | 7.877 | 119.893 | 62.603 | 4.322 | | 1.738 | | QQδ 0.807 |
| **768** | **Q** | 7.802 | 119.419 | 55.532 | 4.437 | | 1.918/1.813 | 2.191 | |
| **769** | **K** | 7.619 | 116.117 | 57.040 | 4.182 | 33.064 | 1.841 | | |
| **770** | **W** | 8.023 | 114.881 | 57.216 | 4.607 | | 3.411 | | Hε1 10.512<br>Nε1 123.999 |
| **771** | **E** | 8.408 | 119.529 | 59.912 | 3.865 | 29.285 | 2.080 | | |
| **772** | **D** | 8.148 | 118.450 | 56.963 | 4.354 | 40.357 | 2.677/2.293 | | |
| **773** | **W** | 7.932 | 120.979 | 61.338 | 4.352 | 29.151 | 3.537/3.300 | | Hδ1 7.263<br>Hε1 10.424<br>Nε1 123.126 |
| **774** | **V** | 8.282 | 118.522 | 61.411 | 3.626 | | 2.131 | 1.052/0.938 | |
| **775** | **R** | 8.097 | 119.012 | | 4.231 | | | | |
| **776** | **W** | 7.865 | 120.025 | 61.455 | 4.288 | | 3.389 | | Hε1 10.511 |
| **777** | **I** | 8.493 | 118.531 | 61.471 | 3.648 | | 2.050 | 1.484/0.988 | |
| **778** | **G** | 8.073 | 104.570 | 46.587 | 3.853 | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **779** | **N** | 7.412 | 116.699 | 53.260 | 4.826 | 39.672 | 2.756/2.678 | | |
| **780** | **I** | 7.800 | 121.083 | 60.065 | 3.783 | | 2.101 | 1.663 | Qδ1 0.904 |
| **781** | **P** | | | | | | | 2.042 | Qδ 3.854 |
| **782** | **Q** | 7.946 | 119.632 | 65.547 | 3.985 | | 2.021 | | |
| **783** | **Y** | 7.939 | 119.882 | 57.352 | 4.260 | | 3.154 | | Qε 7.149<br>Cε1 92.486 |
| **784** | **L** | 7.738 | 116.447 | 57.971 | 4.134 | | 1.862 | | QQδ 0.872 |
| **785** | **K** | | | | | | | | |
| **786** | **M** | | | | | | | | |

**Figure S2: A)** FPLC chromatogram obtained using: binding buffer (10 mM Guanidine, 0,5 M NaCl, 20 mM Tris-HCl and 15mM Imidazole) and elution buffer (10 mM Guanidine, 0,5 M NaCl, 20 mM Tris-HCl and 50 mM Imidazole). The protein was purified using His-Trap™ HP column at 1 mL/min and AKTA purifier system. **B)** HPLC chromatogram, obtained using gradient: 10-40 B in 20 min then 40-70 B in 30 min, Buffer A: water + TFA 0.1%, Buffer B: Acetonitril + TFA 0.1%; the protein was purified using C18 Phenomenex™ column at 2 mL/min. **C)** SDS-PAGE of FPLC fractions (4-5), after cleavage (6) and HPLC fractions (7 KSI - 8 isotope labeled gp36 CHR-MPER).