Angewandte Chemie
Eine Zeitschrift der Gesellschaft Deutscher Chemiker

Supporting Information

Solid-state NMR Study of the YadA Membrane-Anchor Domain in the Bacterial Outer Membrane

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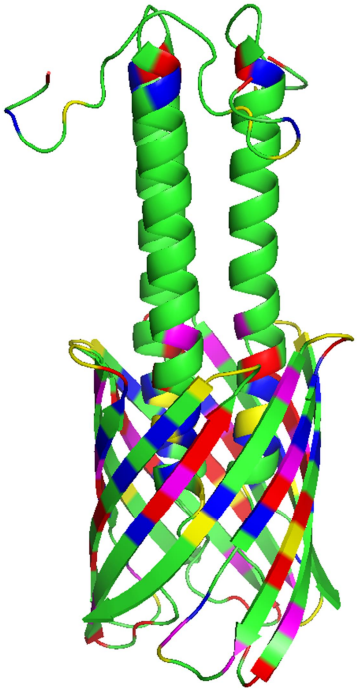
Supplementary Information

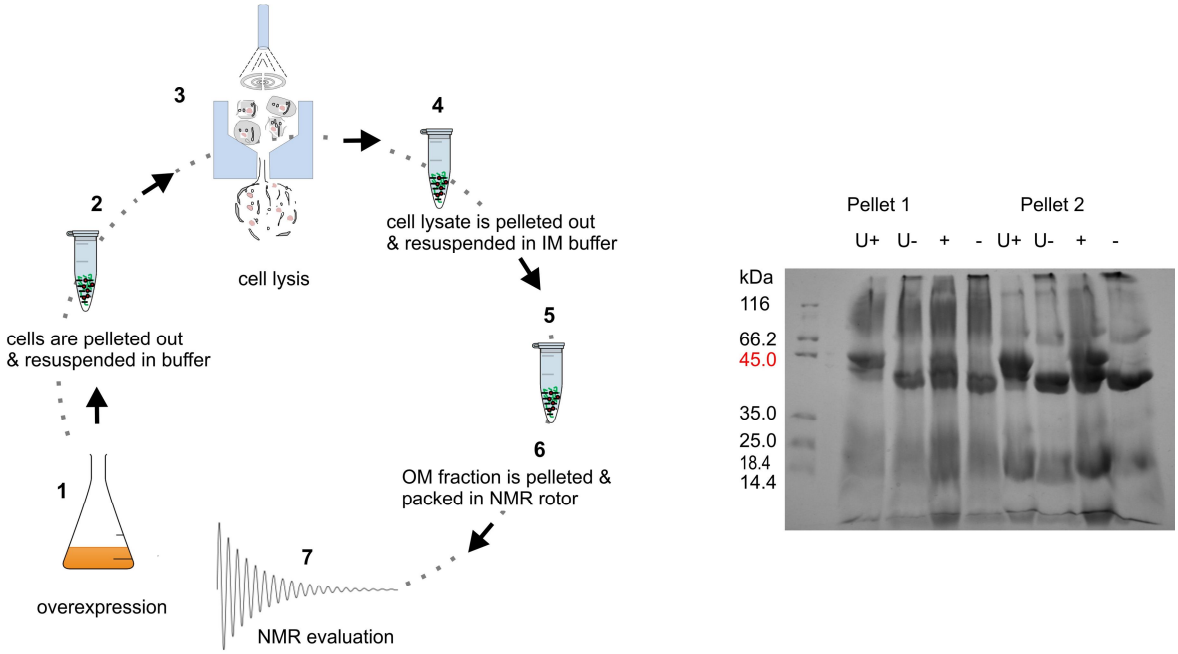


Supplementary Figure 1: GVAS labeled YadA-M. G, V, A, and S are colored red, magenta, blue, and yellow, respectively. Most Gly and Ala residues in YadA-M face the pore lumen to presumably minimize resistance to the passenger domain during autotransport, Val sidechains point tohe lipid membrane and occupy the exterior wall of the ß-barrel, and Ser residues are exclusively present in the ß-turn regions

Preparation of Yad-M in the outer membrane for solid-state MAS NMR

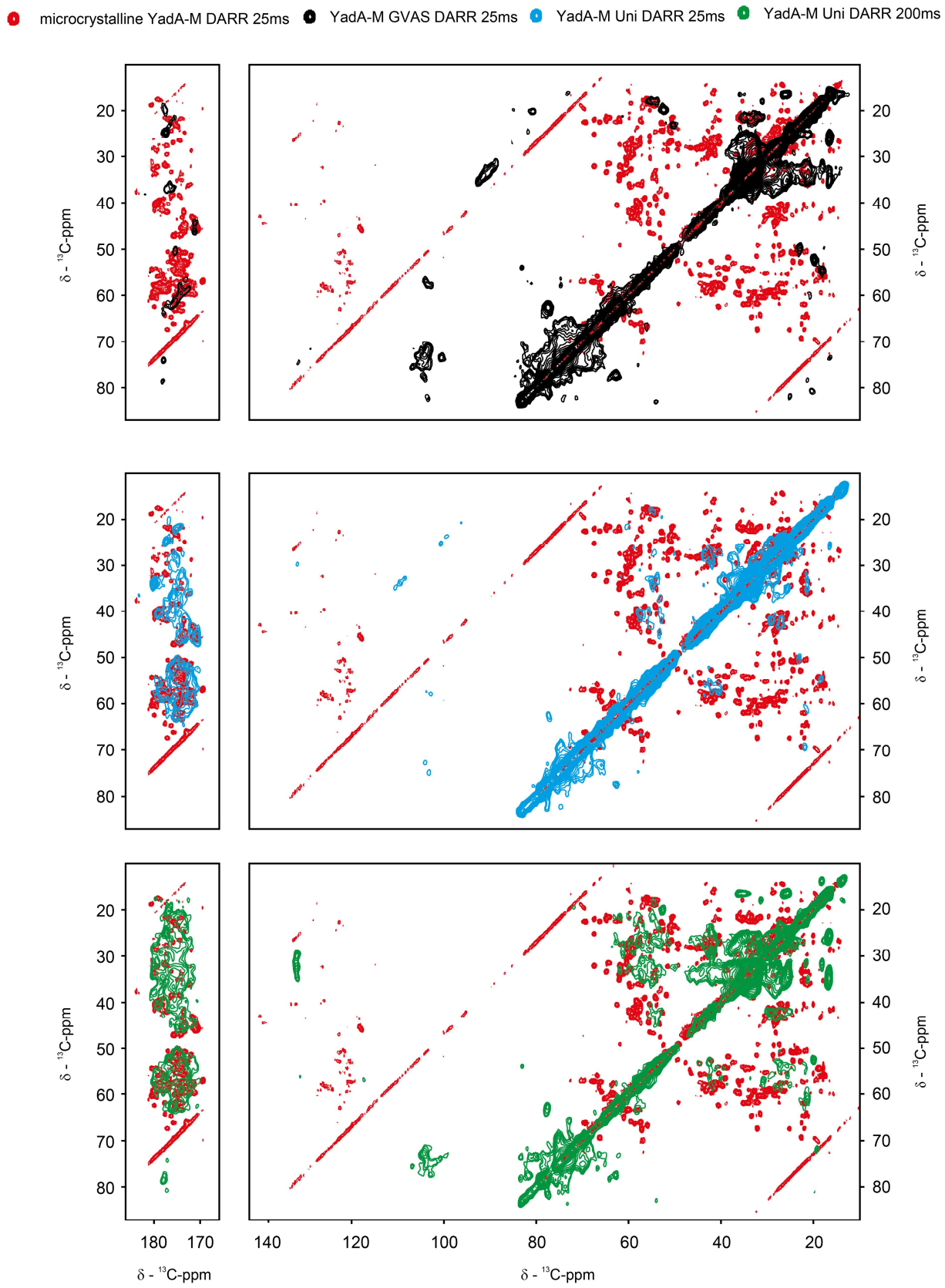
The following YadA-M samples were prepared for solid-state MAS NMR studies, directly in bacterial outer membranes: (1) a uniformly ^{13}C, ^{15}N labeled sample; (2) a selectively ^{13}C labeled ñGVASò YadA-M, where only glycines, valines, alanines and serines were labeled. YadA-M was overexpressed in freshly transformed $CaCl_2$-competent *Escherichia coli* BL21Omp8 cells following the established protocol [1]. Cells were grown in Luria broth supplemented with 60 µg/ml Carbenicillin. The incubation was carried out in a shaker with 170 rpm at 30ºC. When the OD_{600} reached 0.4, the cells were harvested by gentle centrifugation (2000 g) for 20 minutes at 18ºC. The pellets were resuspended in M9 medium to achieve both uniformly (YadA-M Uni) and GVAS labeled (YadA-M GVAS) samples. For producing the uniformly labeled sample, we resuspended the cells in M9 medium which was enriched with ^{13}C-labeled glucose and ^{15}N-labeled ammonium chloride. The YadA-M

GVAS sample was produced by a reverse labeling strategy by resuspending the cell pellet in M9 medium which was supplemented with 4 g ^{13}C-labeled glucose and sixteen unlabeled amino acids, except for G,V,A and S. The cells were incubated for 15 minutes at 37ºC before inducing them with 0.2µg/ml anhydrotetracycline (AHTC). The culture was shaken overnight with 170 RPM at 30ºC and the cells were subsequently harvested at 4500 *g* for 30 minutes at 4ºC, followed by resuspension in lysis buffer (PBS supplemented with 1mM $MgCl_2$ and DNase). The cells were lysed with a homogenizer (Avestin Emulsiflex C3) for 15 minutes and pelleted at 4500 *g* to remove unlysed cells. The supernatant was then spun in a Beckmann SW 45Ti rotor at 100000 *g* for 30 minutes at 8ºC to collect the membrane fraction. The inner membrane (IM) fractions were removed by solubilizing the pellet in IM solubilization buffer (1% (w/v) N-lauroylsarcosine in 20 mM Tris, pH 8.0) [2]. The outer membrane (OM) pellet was washed with PBS buffer six times before packing it in the NMR rotor.

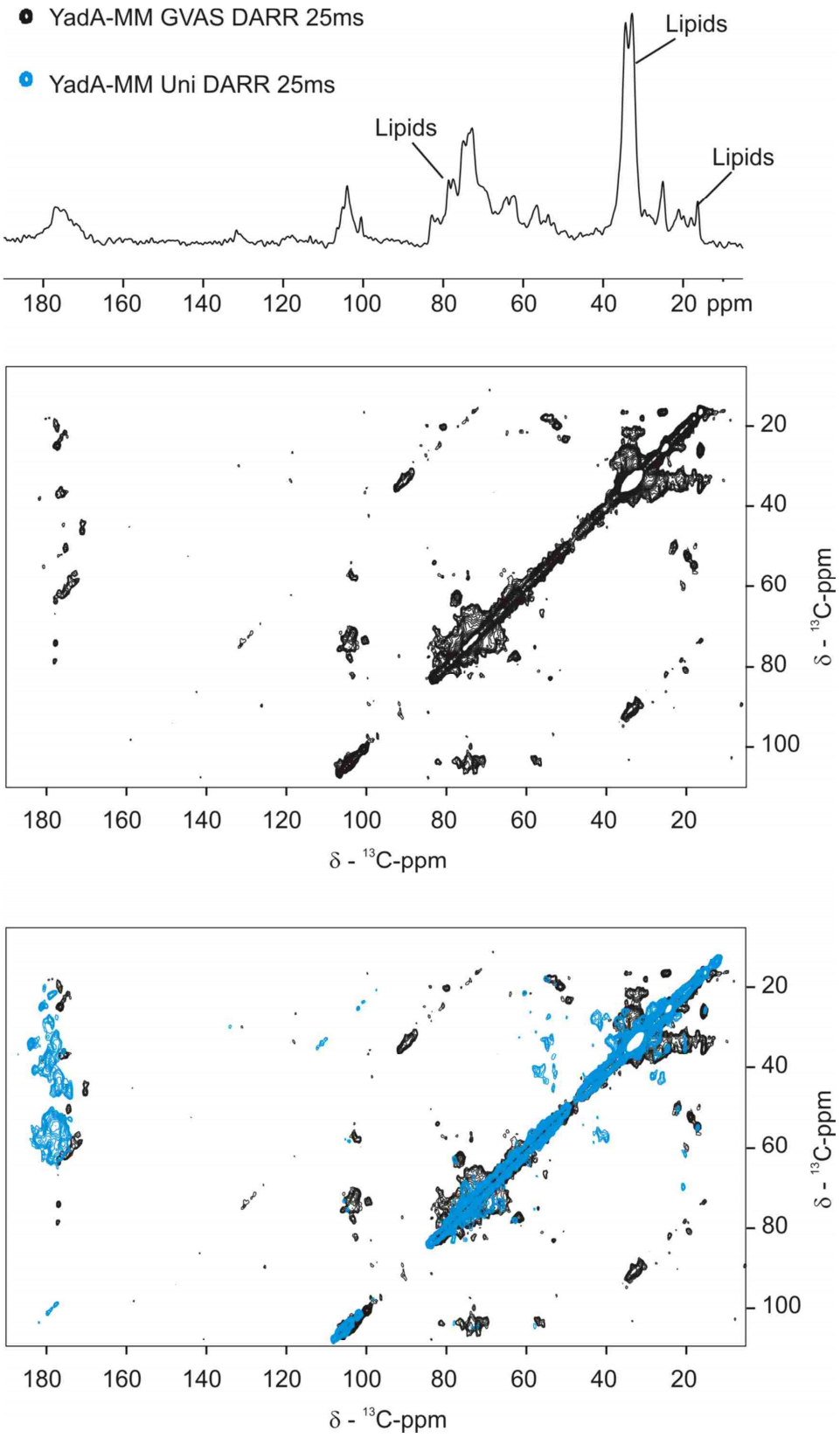


Supplementary Figure 2: Preparation of membrane-bound YadA-M samples for solid-state MAS NMR studies; (left) A schematic representation of major steps from overexpression to NMR measurement of YadA-M (right) SDS-PAGE for YadA-M in native outer membranes (OM). YadA-M was overexpressed and purified according to the reference [1]. After discarding the inner-membrane (IM) debris, the OM pellet (Pellet 1) was resuspended and washed. The supernatant was ultracentrifuged to obtain the pellet (Pellet 2). The SDS-PAGE shows the presence of YadA-M in both pellet fractions. In fact, the amount of YadA is presumably higher in the supernatant fraction (Pellet 2). The stability of YadA was checked by heating it with and without 1M Urea. YadA-M remains an intact trimer under these conditions [1].

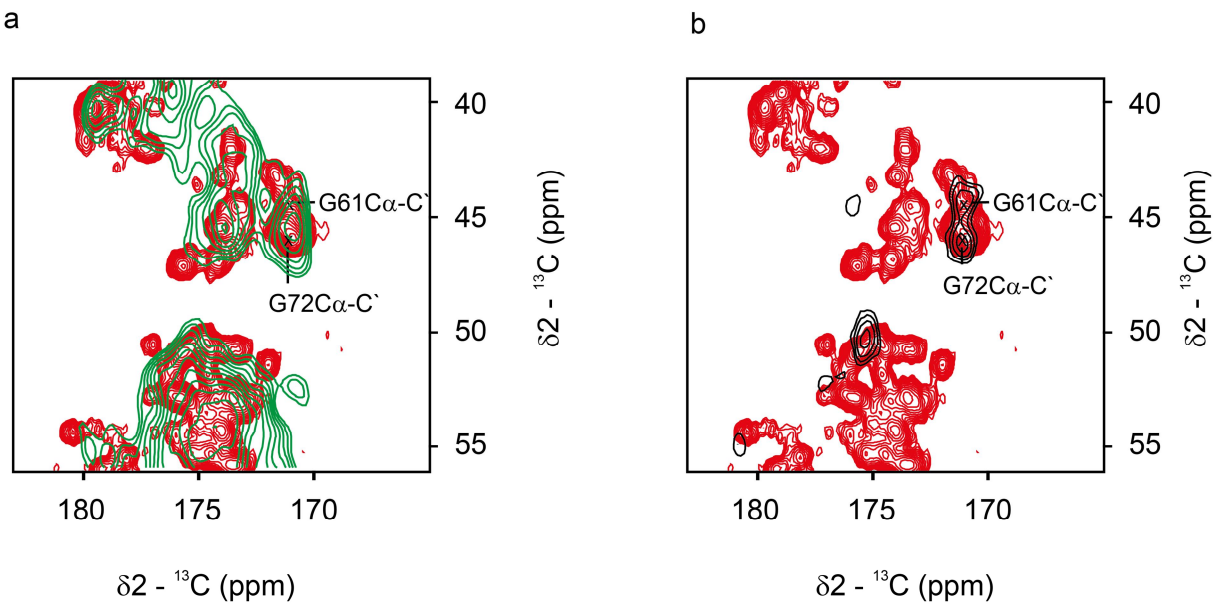
SDS-PAGE shows roughly 70% YadA-M protein inside the OM pellet. We adopted two strategies to get the pure OM pellet. First, after each wash with PBS the OM suspension was centrifuged gently at 5000 rpm for 15 minutes. Most of the protein does not go into this pellet (pellet 1) and results in a turbid supernatant. The supernatant after each wash was collected in a container and later was subjected to ultracentrifugation at 30,000 rpm for 30 minutes to get the OM pellet (Pellet 2). As can be seen in SI Figure 1, pellet 2 contains more YadA-M. Hence pellet 2 was further washed thoroughly with large amounts of PBS followed by ultracentrifugation. The SDS results show little difference in purity and stability of YadA-M. The expression strain used in this study, BL21Omp8 [3], lacks most major outer membrane proteins, and thus contributes to the purity of the sample. Samples from pellet 2 were packed in thin-walled 3.2 mm and standard 3.2 mm MAS rotors using a home-made filling device in an ultracentrifuge for NMR spectra acquisition.



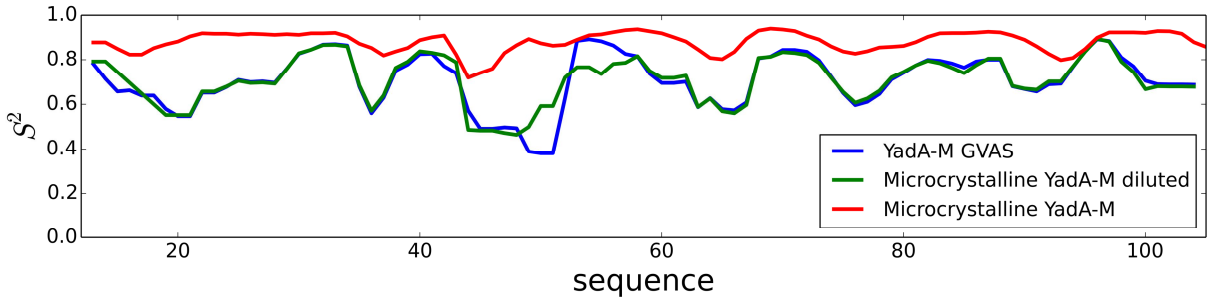
Supplementary Figure 3: Comparison of 2D ^{13}C DARR spectra from microcrystalline YadA-M (red contours) with GVAS YadA-M in membranes (black contours) and uniformly labeled YadA-M in membranes (cyan and green contours).



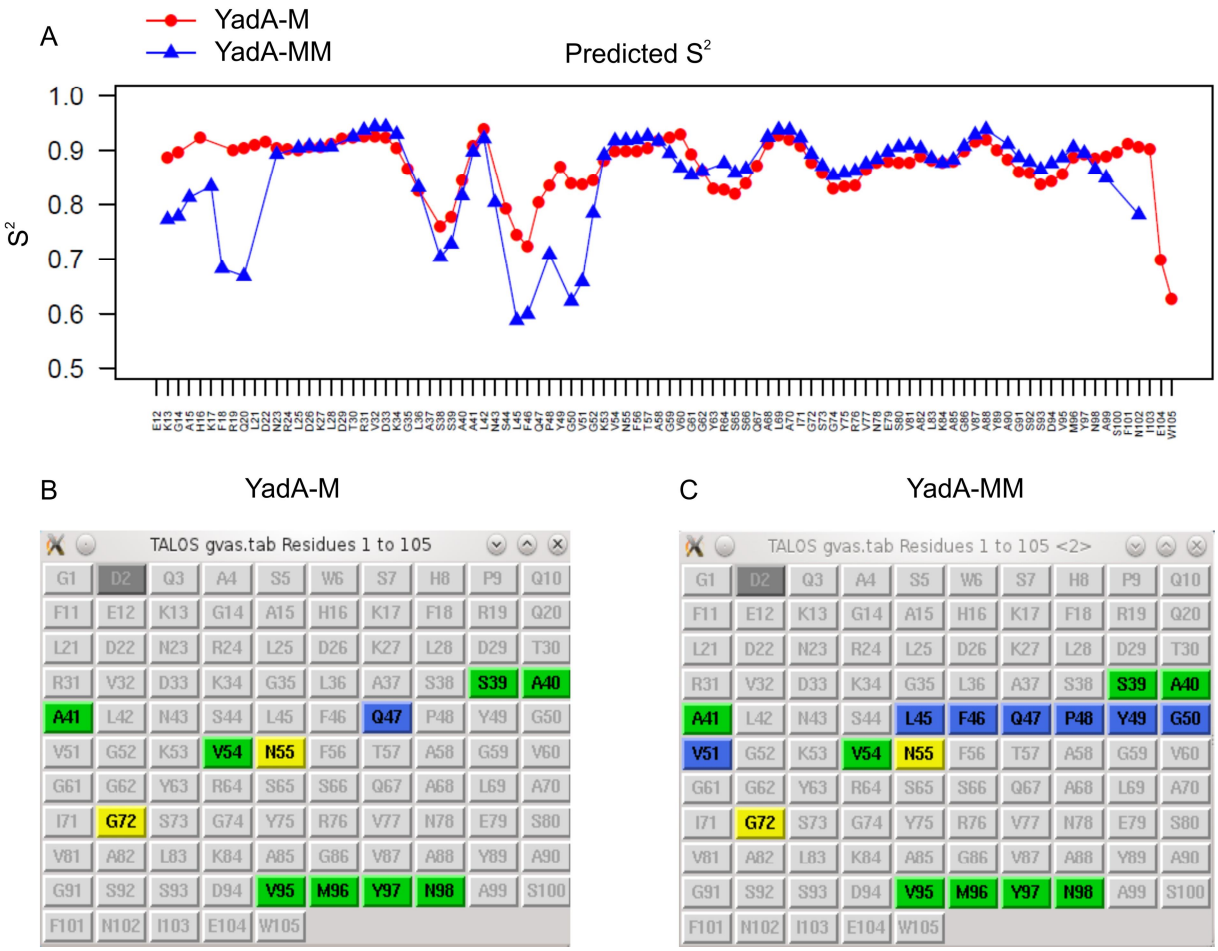
Supplementary Figure 4: Spectral overlay of contour plots recorded on YadA-MM GVAS (black contours) and uniformly labeled YadA-MM (cyan contours). Both spectra were recorded under similar conditions with 25ms DARR mixing scheme. The signals from lipids and Gly, Val, Ala, Ser residues overlap in both spectra.



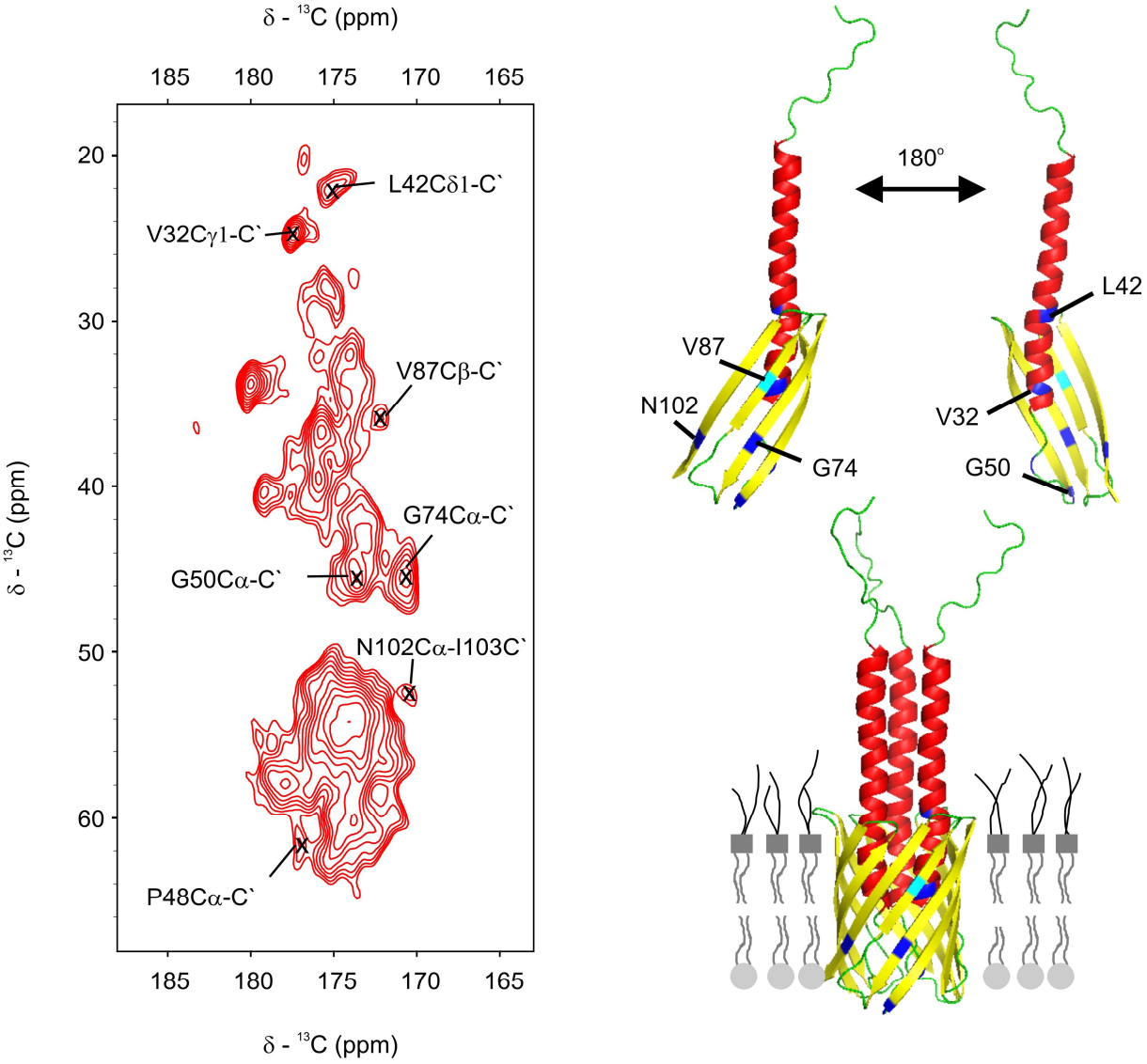
supplementary Figure 5: Overlay of microcrystalline YadA-M contour plots (red) with membrane bound uniformly labeled (a) and GVAS labeled (b) YadA-M samples.



Supplementary Figure 6: Order parameter analysis. Residue-wise order parameters were predicted using TALOS+ based on the chemical shifts of YadA-M GVAS (blue) and microcrystalline YadA-M (red). Microcrystalline YadA-M appears to be less mobile according to this analysis. However, this result has to be taken with some caution as the differences in flexibility might result from differences in the completeness of the chemical shift assignment. If the chemical shifts that are missing in YadA-M GVAS compared to microcrystalline YadA-M are removed from the list of chemical shifts of microcrystalline YadA-M, the order parameters turn out to be very similar (green curve). Nevertheless, the incompleteness of the YadA-M GVAS shifts itself clearly indicates that some regions of YadA-M are more flexible in native membrane than in the micro-crystalline environment.



Supplementary Figure 7: TALOS Plus predictions for YadA-MM reveal a higher mobility in the L45-V51 region. This is the random coil which connects the beta sheet with the N-terminal alpha helix. The secondary structure prediction effeciency of TALOS+ depends upon backbone ^{15}N and ^{13}C chemical shifts of triplets. Missing ^{15}N chemical shifts and sparse ^{13}C chemical shifts result into poor secondary structure prediction (panel B). However, when TALOS+ is run together with microcrystalline YadA-M chemical shifts, it shows the residues from L45-V51 extremely dynamic (panel C). Amino-acid residues highlighted in blue are predicted to be more dynamic.



Supplementary Figure 8: The amino acid residues facing the pore lumen generally show stronger cross peaks as compared to those facing the highly dynamic membrane environment. The contour plots are taken from 2D-DARR 25 ms spectrum recorded on uniformly labeled YadA-M. Except for V87 all other assignments are from lumen-facing residues. An obvious difference in peak intensities can be observed between V87 and other residues. The positions of the residues are highlighted in monomer and trimer structures of YadA-M on right side of the Figure.

The following table gives a summary of conditions under which various solid-state MAS NMR data were recorded on uniformly and GVAS labelled YadA-MM (YadA membrane anchor domain in outer membranes).

No.	Sample	Type of experiment	Mixing time (ms)	MAS (kHz)	Temperature (K)	Field strength (MHz)	No. of scans	No. of scans in F1	Direct acquisition time (ms)	Indirect acquisition time (ms)	Total Experiment time (days)
1	YadA-GVAS	DARR[4]	25	10	275	700	160	600	20	8.5	3 days 2 hours
2	YadA-GVAS	DARR	100	13.33	275	700	160	504	20	6.3	2 days 14 hours
3	YadA-GVAS	DARR	200	10	260	600	96	532	19	8.3	1 day 16 hours
4	YadA-GVAS	PDSD[5]	300	10	260	600	160	700	19	8.75	3 days 15 hours
5	YadA-Uni	DARR	25	13.33	275	700	160	520	20	6.5	2 days 17 hours
6	YadA-Uni	DARR	200	10	275	700	160	520	20	6.5	2 days 17 hours
7	YadA-Uni	PDSD	300	10	260	700	160	750	20	9.3	3 days 17 hours
8	YadA-Uni	PDSD	500	10	260	700	160	352	15	8.7	1 day 20 hours
9	YadA-Uni	DARR-direct ^{13}C acquired	25	10	275	700	80	1012	20	12.6	2 days 20 hours
10	YadA-Uni	DARR-direct ^{13}C acquired	100	10	275	700	96	624	20	7.8	2 days 2 hours
11	YadA-Uni	DARR-direct ^{13}C acquired	200	10	275	700	80	1012	20	12.6	2 days 20 hours

Supplementary Table 1: List of two-dimensional ^{13}C-^{13}C correlation spectra recorded on selectively labeled GVAS and uniformly labeled YadA-M outer membrane samples (YadA-MM). In experiments 9-11, direct carbon excitation was used followed by DARR [4] mixing. The inter-scan delay between such experiments was kept 3 seconds. For all other experiments this delay was kept relatively short i.e. 2.8 seconds.

Supplementary Table 2: Solid-state MAS NMR ^{13}C chemical shifts of YadA membrane anchor domain in bacterial outer membranes (YadA-M).

Residue	C`	Cα	Cβ	Cδ	Cδ1	Cδ2	Cε	Cγ	Cγ1	Cγ2
K13L	173.9	57.64	-	-	-	-	-	21.22	-	-
G14L	171.1	-	-	-	-	-	-	-	-	-
A15L	-	54.08	-	-	-	-	-	-	-	-
K17L	-	60.23	33.30	-	-	-	-	-	-	-
F18L	-	57.83	38.69	-	-	-	-	-	-	-
R19L	-	-	-	42.96	-	-	-	-	-	-
Q20L	177.9	-	-	180.0	-	-	-	33.74	-	-
N23L	-	56.39	38.90	-	-	-	-	-	-	-
R24L	-	-	-	-	-	-	-		-	-
L25L	179.0	58.48	43.12	-	-	-	-	27.74	-	-
D26L	-	-	40.08	-	-	-	-	-	-	-
K27L	-	-	32.32	-	-	-	-	-	-	-
L28L	-	58.27	42.08	-	26.81	23.45	-	27.97	-	-
T30L	175.7	66.10	69.93	-	-	-	-	-	-	21.92
R31L	180.3	-	31.81	-	-	-	-	28.24	-	-
V32L	177.3	67.16	-	-	-	-	-	-	24.94	21.83
D33L	179.2	58.23	-	-	-	-	-	-	-	-
K34L	-	61.17	33.70	27.37	-	-	-	-	-	-
L36L	-	57.45	-	-	-	-	-	-	-	-
S38L	173.0	58.78	-	-	-	-	-	-	-	-
S39L	178.7	57.95	63.10	-	-	-	-	-	-	-
A40L	179.9	54.24	17.94	-	-	-	-	-	-	-
A41L	179.5	55.18	17.78	-	-	-	-	-	-	-
L42L	175.2	62.72	-	-	21.91	-	-	26.81	-	-
N43L	176.1	53.33	-	-	-	-	-	-	-	-
L45L	-	55.32	40.66	-	-	-	-	25.56	-	-
F46L	177.6	57.57	40.11	-	-	-	-	-	-	-
P48L	177.1	61.59	32.41	49.99	-	-	-	27.24	-	-

G50L	173.6	45.55	-	-	-	-	-	-	-	-
V51L	177.3	-	31.25	-	-	-	-	-	21.45	-
G52L	-	45.92	-	-	-	-	-	-	-	-
K53L	175.3	54.08	-		-	-	42.02	25.13	-	-
V54L	171.9	59.74	36.68	-	-	-	-	-	21.58	-
N55L	173.5	53.28	44.80	-	-	-	-	-	-	-
F56L	171.7	57.94	-	-	-	-	-	-	-	-
T57L	-	56.97	71.94	-	-	-	-	-	-	19.32
A58L	175.3	50.34	23.18	-	-	-	-	-	-	-
G59L	-	45.08	-	-	-	-	-	-	-	-
V60L	175.0	-	-	-	-	-	-	-	-	22.54
G61L	171.4	44.30	-	-	-	-	-	-	-	-
G62L	-	44.90	-	-	-	-	-	-	-	-
R64L	176.9	59.09		-	-	-	-	-	-	-
S65L	-	57.59	63.34	-	-	-	-	-	-	-
S66L	173.1	59.36	-	-	-	-	-	-	-	-
A68L	173.2	50.87		-	-	-	-	-	-	-
L69L	-	53.03	-	-	-	-	-		-	-
A70L	175.2	50.47	-	-	-	-	-	-	-	-
I71L	173.5	59.38	43.16	-	13.67	-	-	-		18.20
G72L	171.1	46.21	-	-	-	-	-	-	-	-
S73L	173.0	57.02	66.46	-	-	-	-	-	-	-
G74L	170.6	45.49	-	-	-	-	-	-	-	-
Y75L	-	-	43.12	-	-	-	-	-	-	-
R76L	-	55.26	-	43.26	-	-	-	-	-	-
V77L	-	64.61	32.99	-	-	-	-	-	21.30	-
N78L	174.6	-	38.41	-	-	-	-	176.5	-	-
E79L	177.3	59.05	28.89	183.4	-	-	-	36.36	-	-
S80L	174.0	60.54	-	-	-	-	-	-	-	-
V81L	174.2	-	35.26	-	-	-	-	-	21.43	-
A82L	174.4	50.64	-	-	-	-	-	-	-	-
L83L	175.4	55.02	46.53	-	26.35	-	-	27.95	-	-

K84L	171.8	57.19	-	29.61	-	-	40.87	-	-	-
A85L	176.0	53.01	-	-	-	-	-	-	-	-
G86L	-	46.48	-	-	-	-	-	-	-	-
V87L	172.2	59.88	36.04	-	-	-	-	-	20.97	21.63
A88L	-	51.25	27.24	-	-	-	-	-	-	-
A90L	-	50.83	22.73	-	-	-	-	-	-	-
G91L	173.4	45.09	-	-	-	-	-	-	-	-
S92L	-	56.14	66.45	-	-	-	-	-	-	-
S93L	-	-	63.31	-	-	-	-	-	-	-
D94L	-	54.10	41.03	-	-	-	-	-	-	-
V95L	174.4	61.88	34.40	-	-	-	-	-	-	21.29
M96L	174.6	54.38	39.74	-	-	-	18.44	33.05	-	-
Y97L	173.1	56.00	42.46	-	-	-	-	-	-	-
N98L	173.2	52.89	41.43	-	-	-	-	-	-	-
A99L	-	52.32	22.72	-	-	-	-	-	-	-
N102L	-	52.55	-	-	-	-	-	176.8	-	-
I103L	170.5	-	-	-	-	-	-	-	28.36	-

The increased dynamics in the membranes and low sensitivity of YadA-M results into very weak cross peaks even for backbone nuclei. A complete fingerprint pattern was rarely observed except those from few alanines and glycines. Hence, most most amino-acid residues were only partially assigned on the basis of residue specific and sequential contacts. Almost 75% of the primary sequence (excluding first 15 strep-tag residues) were assigned ï most of them having assignments for one or two nuclei.

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