Supplementary Information

Resolving atomic site interactions of the *Y. pestis* outer membrane protein Ail with human serum in the bacterial cell envelope

James E. Kent [a], Lynn M. Fujimoto [a], Kyungsoo Shin [a], Chandan Singh [a,d], Yong Yao [a], Sang Ho Park [b], Stanley J. Opella [b], Gregory V. Plano [c], and Francesca M. Marassi [a] *

[a]Cancer Center, Sanford Burnham Prebys Medical Discovery Institute, 10901 North Torrey Pines Road, La Jolla CA, 92037, USA.

[b]Department of Chemistry and Biochemistry University of California San Diego, La Jolla, CA, 92037, USA.

[c]Department of Microbiology and Immunology, University of Miami Miller School of Medicine, Miami, FL 33101, USA

[d]Present address: Department of Biochemistry, Institute of Science, Banaras Hindu University (BHU), Varanasi, Pin-221005

[*]Corresponding author: fmarassi@sbp.edu

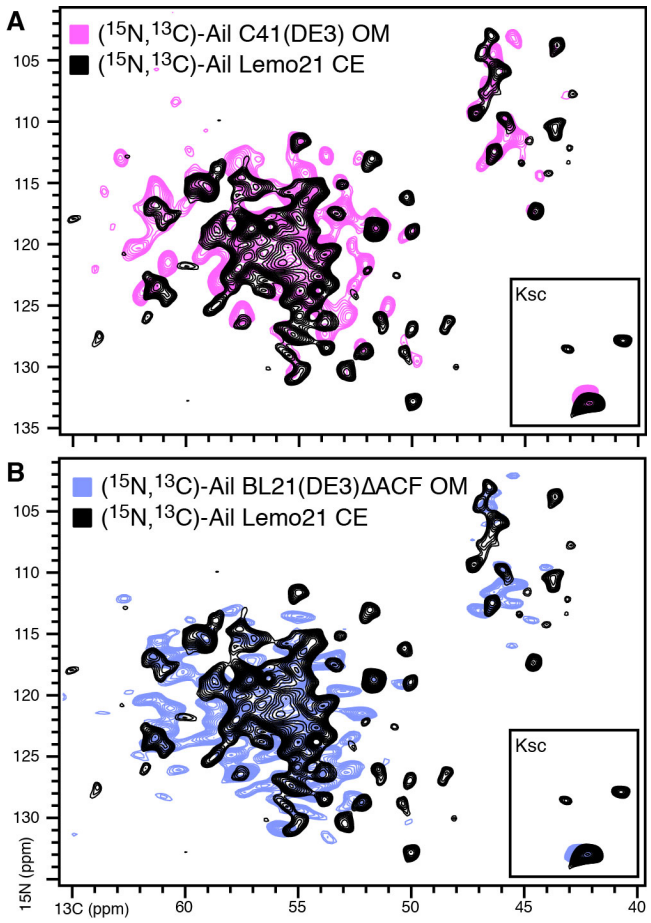

Figure S1. 2D $^{15}N/^{13}C$ NCA solid-state NMR spectra of Ail in *E. coli* cell envelopes (CE), *E. coli* outer membranes (OM) and liposomes. (A, B) NMR spectra were acquired for $^{15}N,^{13}C$-Ail in *E. coli* CE from Lemo21(DE3) cells (black) or *E. coli* OM from C41(DE3) cells (pink), *E. coli* OM from BL21(DE3)ΔACF cells (violet). NMR spectra were recorded at 750 MHz, 7°C, with a MAS rate of 11 kHz. Inset box denotes folded-in peaks from Lys side chains (Ksc).

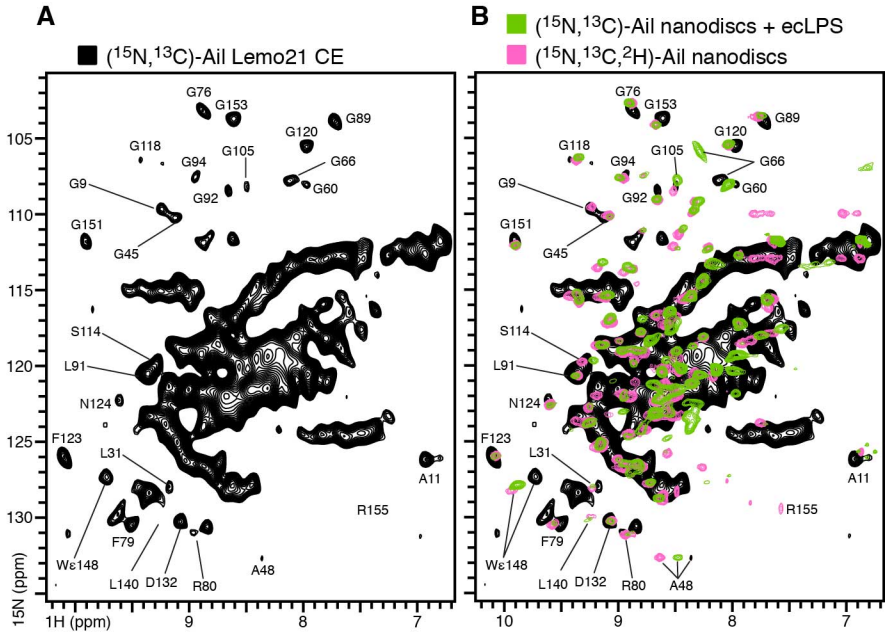

Figure S2. Solid-state NMR CP-HSQC and solution NMR HSQC $^{1}H/^{15}N$ 2D spectra of Ail in *E. coli* cell envelopes (CE) and nanodiscs. Solid-state NMR CP-HSQC spectra (black) were acquired for (^{15}N,^{13}C)-Ail in *E. coli* CE from Lemo21(DE3) cells, at 900 MHz, 30°C, with a MAS rate of 60 kHz and 1,600 transients. Solution NMR HSQC spectra were acquired for purified Ail uniformly labeled with ^{15}N, ^{13}C and ^{2}H in lipid nanodiscs (pink), or uniformly labeled with ^{15}N and ^{13}C in nanodiscs reconstituted with *E. coli* rough-type LPS (green). The solution NMR spectra of Ail nanodiscs have been described previously [14, 19]

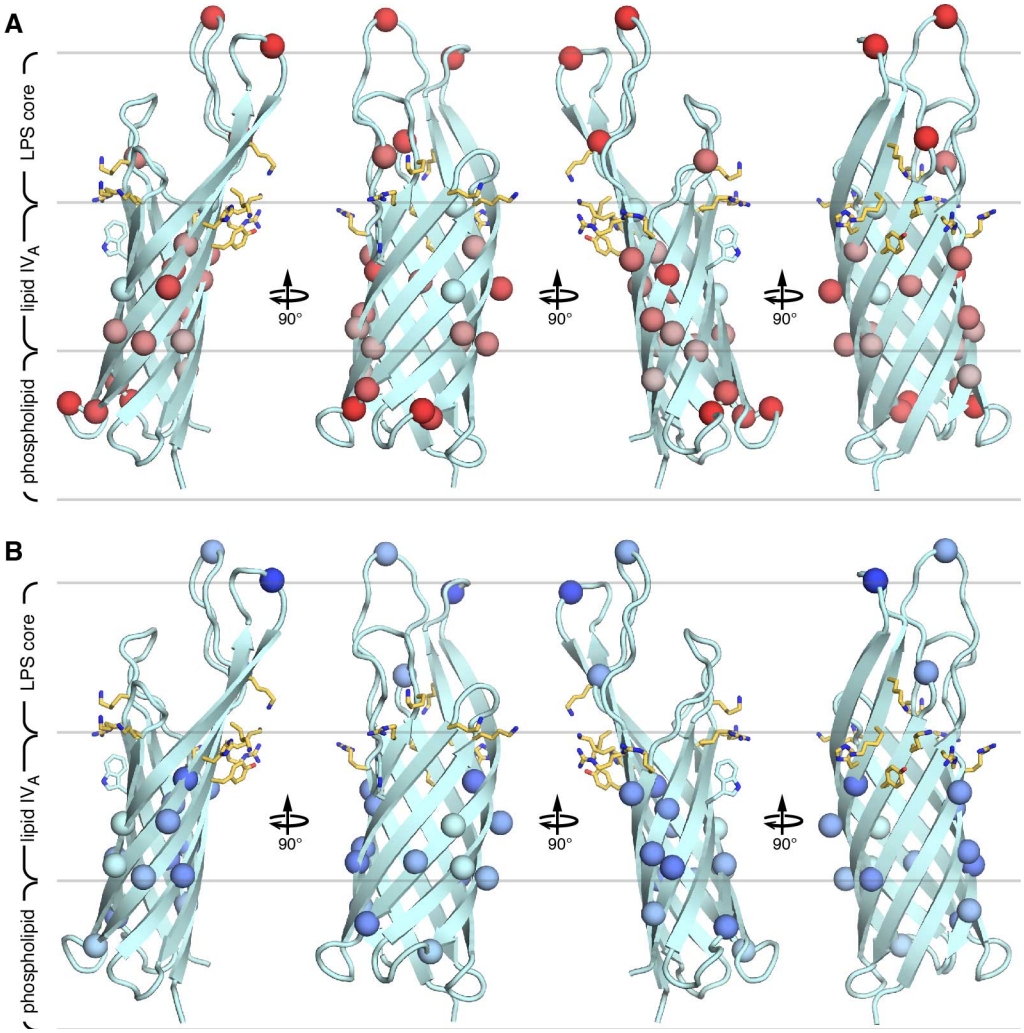

Figure S3. Structural model of Ail embedded in the *Y. pestis* outer membrane showing perturbations associated with the cell envelope environment or interactions with NHS. Sidechains forming two clusters of LPS-recognition motifs are shown as yellow sticks. The boundaries of the outer membrane phospholipid and LPS layers are marked (gray lines). Residue numbers, from E1 to F156, corresponds to the mature sequence of Ail. **(A)** Spheres denote resolved and assigned amide N atoms that undergo $^{1}H/^{15}N$ chemical shift perturbations from 0 ppm (cyan) to 0.15 ppm (red), between the cell envelope and liposome environments. **(B)** Spheres denote resolved and assigned amide N atoms that undergo $^{1}H/^{15}N$ chemical shift perturbations from 0 ppm (cyan) to 0.20 ppm (blue), in the presence of NHS.

Table S1. Resolved $^{1}H/^{15}N$ NMR signals with tentative assignments derived by spectral comparison with the solid-state and solution NMR spectra of purified Ail reconstituted in liposomes or nanodiscs (18, 19). Missing peaks are denoted by the letter "X". The combined difference (ΔHN) of amide ^{1}H and ^{15}N chemical shifts was calculated as: $\Delta HN = [(\Delta H)^2+(\Delta N/5)^2]^{1/2}$.

18. Yao Y, Dutta SK, Park SH, Rai R, Fujimoto LM, Bobkov AA, Opella SJ, Marassi FM (2017) **High resolution solid-state NMR spectroscopy of the Yersinia pestis outer membrane protein Ail in lipid membranes**. *J Biomol NMR* 67, 179-190 (PMC5490241).

19. Dutta SK, Yao Y, Marassi FM (2017) **Structural Insights into the Yersinia pestis Outer Membrane Protein Ail in Lipid Bilayers**. *J Phys Chem B* 121, 7561-7570 (PMC5713880).

Ail residue	liposomes		CE		CE + NHS		lipo VS CE	CE ± NHS
	^{1}H (ppm)	^{15}N (ppm)	^{1}H (ppm)	^{15}N (ppm)	^{1}H (ppm)	^{15}N (ppm)	ΔHN (ppm)	ΔHN (ppm)
9G	9.139	109.788	9.229	109.669	9.123	109.485	0.093	0.113
11A	6.849	126.575	6.931	126.083	6.847	126.337	0.128	0.098
23D	8.141	114.416	8.213	114.070	8.184	114.713	0.100	0.132
28G	8.736	111.021	8.809	111.373	8.731	111.717	0.101	0.104
45G	9.071	110.066	9.115	110.230	9.000	110.325	0.055	0.116
48A	8.324	132.972	8.358	132.585	X	X	0.085	—
60G	X	X	7.966	108.059	8.035	107.870	—	0.078
66G	X	X	8.110	107.786	8.186	107.972	—	0.085
76G	8.852	102.744	8.874	103.164	8.921	103.362	0.087	0.061
80R	8.810	131.135	8.833	130.502	8.800	130.396	0.129	0.039
81I	8.194	126.515	X	x	X	X	—	—
82N	7.837	112.360	X	x	X	X	—	—
89G	7.709	103.532	7.714	103.901	7.712	103.898	0.074	0.002
92G	8.567	108.949	8.649	108.458	8.564	108.557	0.128	0.088
94G	8.937	107.875	8.937	107.534	X	X	0.068	—
105G	X	X	8.487	108.189	8.487	107.165	—	0.205
118G	9.327	106.444	9.415	106.430	9.389	106.433	0.088	0.026
120G	7.969	105.521	7.967	105.562	7.945	105.220	0.008	0.072
123F	10.086	126.487	10.085	125.922	10.158	125.454	0.113	0.119
124N	X	X	9.604	122.208	X	X	—	—
131I	8.830	124.461	8.868	123.959	8.887	124.103	0.107	0.035
132D	9.013	130.121	9.068	130.170	X	X	0.056	—
151G	9.864	112.073	9.894	111.808	9.758	111.882	0.061	0.137
153G	8.630	103.914	8.602	103.716	8.641	103.971	0.048	0.064

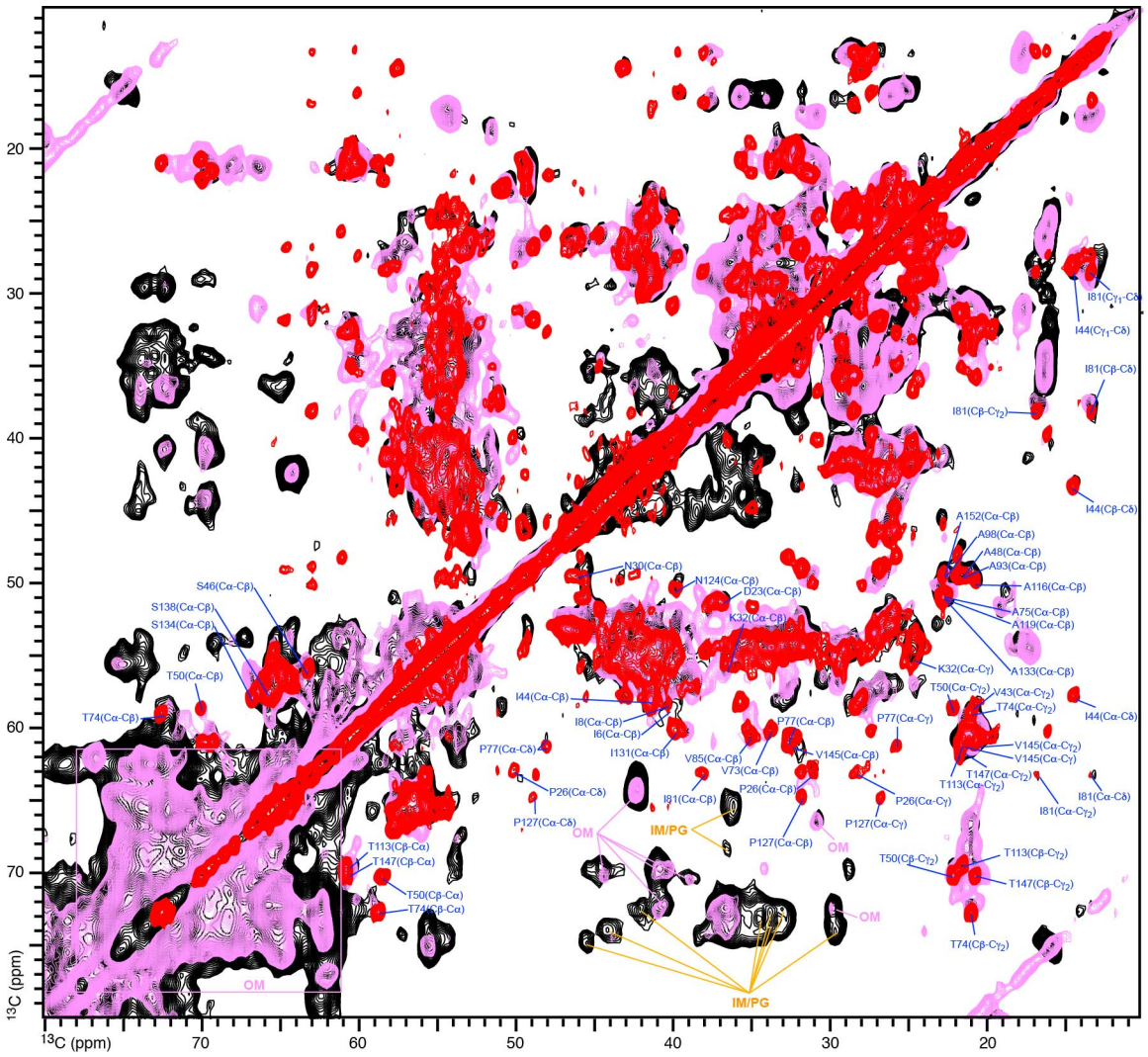

Figure S4. 2D PDSD ^{13}C-^{13}C solid-state NMR spectra of ^{15}N/^{13}C-Ail in *E. coli* cell envelopes (black), *E. coli* outer membranes (pink), or reconstituted liposomes (red). Resolved assigned Ail peaks are marked (blue). Signals from components of the bacterial outer membrane (pink) or inner membrane and peptidoglycan (gold) are tentatively assigned by spectral comparison. Spectra were recorded at 750 MHz, 7°C, with a MAS rate of 11 kHz, and 204 t1 increments with 304 transients for the cell envelope sample, or 400 t1 increments and 32 transients for the outer membrane sample, or 512 t1 increments and 64 transients for the liposome sample.