

Score	Expect	Method	Identities	Positives	Gaps
61.6 bits(148)	2e-16	Compositional matrix adjust.	41/128(32%)	63/128(49%)	10/128(7%)
OmpA1 97	SLKLNVPSSVTFATDQYAITPAFTPLLNDLATTLN-QNPQITASVVG	YTDSTGSAAHNQT	155		
OmpA2 91	SQKITYQADTLDFDKAVLKPAKGKQLDELA	AKIQGMNVEVVVAT-GYTD	IGSDKYNDR	149	
OmpA1 156	LSQNRAQSVVNALAQRGVAANRLSAQGMGASNP	PIADN-ATEAGRAQ-----NRRVEIY	207		
OmpA2 150	LSLRRAQAVKSYLVSKGVPANKVYTEGKGKRN	PVTGNTCKQKNRQLIAC	LAPDRRVEVE	209	
OmpA1 208	LRAPQAAQ	215			
OmpA2 210	VVGTEVQ	217			

Score	Expect	Method	Identities	Positives	Gaps
64.7 bits(156)	5e-18	Compositional matrix adjust.	43/124(35%)	61/124(49%)	11/124(8%)
OmpA1 89	QVTEQPDGSLKLNVPSS-----VTFATDQYAITPAFTPLLNDLATTLNQNPQITASVVG	142			
Pal 48	QVT P LN P+S V F D Y++ + LL A L +PQ + G	102			
OmpA1 143	YTDSTGSAAHNQTLSQNRAQSVVNALAQRGVAANRLSAQGMGASNP	PIADNATEAGRAQNR	202		
Pal 103	NTDERGTSEYNLALGQKRAEAVRRALSLLGVGDAQMEAVSLGKEK	PVALGHDEASWAQNR	162		
OmpA1 203	RVEI	206			
Pal 163	RADL	166			

Score	Expect	Method	Identities	Positives	Gaps
36.6 bits(83)	6e-08	Compositional matrix adjust.	24/82(29%)	39/82(47%)	0/82(0%)
OmpA2 102	FDFDKAVLKPAKGKQLDELA	AKIQGMNVEVVVATGYTD	IGSDKYNDRLSLRRAQAVKSY	161	
Pal 68	FDFD ++ + L + A ++ ++ G TD G+ +YN L +RA+AV+	127			
OmpA2 162	LVSKGVPANKVYTEGKGKRN	PV	183		
Pal 128	LSLLGVGDAQMEAVSLGKEK	PV	149		

Fig S1: BLASTp Pairwise Comparisons of OmpA1, OmpA2, and Pal. Protein sequence FASTA files of OmpA1 (BPSL0999), OmpA2 (BPSL2522), and Pal (BPSL2765) from *Bpm* strain K96243 were obtained from UniProt. Pairwise comparisons of the sequences were performed with the NCBI standard protein BLAST web tool with default parameters.

```

OmpA2      -MNKLSKLAFI---AATAVMAASASASQSVPASR-----QAVNDNWVNGTG 41
OmpA1      -MNTKIATRLSVFALAGALLAGCATQQGTNTAVGTGTGAALGAGIGALAGGGKGAAG 59
Pa1        MMSKKLRLAFAM--LMIGALAACKSGVKLD-----EHA--NQGDA---- 36
           *..      :      . :*.. :      ... .

OmpA2      EWVWMNGTNELCWRDAFWTPATANAKCDGALVAQAPAPAPVAPVAPAITSQKITYQADTL 101
OmpA1      VGALVGGVTGYNWQAIKN-KLAPSAQQTGTQVTEQPD-----GSLKLNVPSSVT 107
Pa1        -----VST---QPNPEN-VAQVTVDPL-----NDPNSPLAKRSVY 67
           . :      : * : *      .      ..

OmpA2      FDFDKAVLKPAGKQKLDELAALKIQGMNVEVVVATGYTDRIGSDKYNDRLSLRRAQAVKSY 161
OmpA1      FATDQYAITPAFTPLLNDLATTLNQNPQITASVVGTYDSTGSAAHNQTLSQNRAQSVVNA 167
Pa1        FDFDSYSVQDQYQALLQQHAQYLKSHQPQRHILIQGNTDERGTSEYNLALGQKRAEAVRRA 127
           * *. :      *:: * ::      * ** *: : * *. .**::*
                               ↑           ↑

OmpA2      LVSKGVPANKVYTEGKGKRNPTGNTCKQKNRKQLIACLAPDRRVEVEVVGTEVQKTTV 221
OmpA1      LAQRGVAANRLSAQGMGASNPIADNATEAG-----RAQNRREIYLRAQAAQ---- 215
Pa1        LSLLGVGDAQMEAVSLGKEKPVALGHDEAS-----WAQNRRLADLVYQQ----- 170
           * ** :: : . * :*: . :      * :*:.:

OmpA2      PAQ 224
OmpA1      --- 215
Pa1        --- 170

```

Fig S2: Clustal Omega Multiple Alignment of OmpA1, OmpA2, and Pa1. Protein sequence FASTA files of OmpA1 (BPSL0999), OmpA2 (BPSL2522), and Pa1 (BPSL2765) from *Bpm* K96243 were obtained from UniProt. Multiple sequence alignment was performed with Clustal Omega. An asterisk (*) denotes a residue that is strictly conserved. A colon (:) indicates the residues have highly similar physiochemical properties. A period (.) indicates that residues have weakly similar properties. Red arrows were overlaid to indicate residues previously identified as being involved in peptidoglycan binding and that are broadly conserved across Gram-negative species.

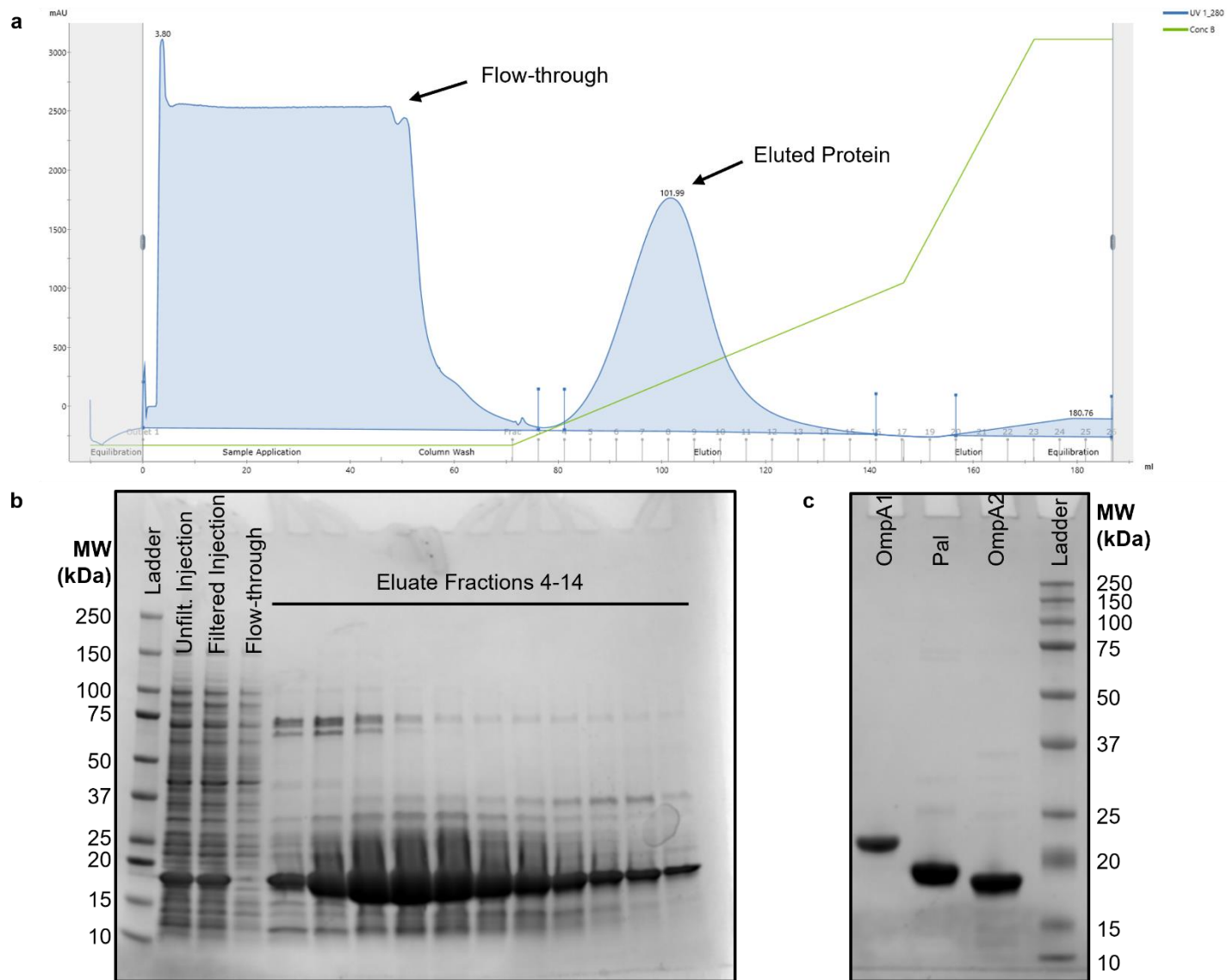


Fig S3: Purification of Recombinant OmpA1, OmpA2, and Pal. *E. coli* harboring plasmids encoding OmpA1, OmpA2, and Pal were induced, pelleted, and lysed as described. Lysate was applied to a Cytiva HisTrap HP column connected to an ÄKTA pure protein purification system. **(a)** Representative chromatogram from the purification of OmpA2. **(b)** SDS-PAGE with Coomassie stain of the OmpA2-containing lysate (pre- and post-filtering), flow-through, and fractions 4 through 14 of the HisTrap HP column eluate containing OmpA2. **(c)** SDS-PAGE with Coomassie stain of fully purified OmpA1, OmpA2, and Pal. ImageJ densitometry indicates a purity of > 95%. Expected molecular weights: OmpA1 = 20.2 kDa, Pal = 17.5 kDa, OmpA2 = 16.3 kDa.

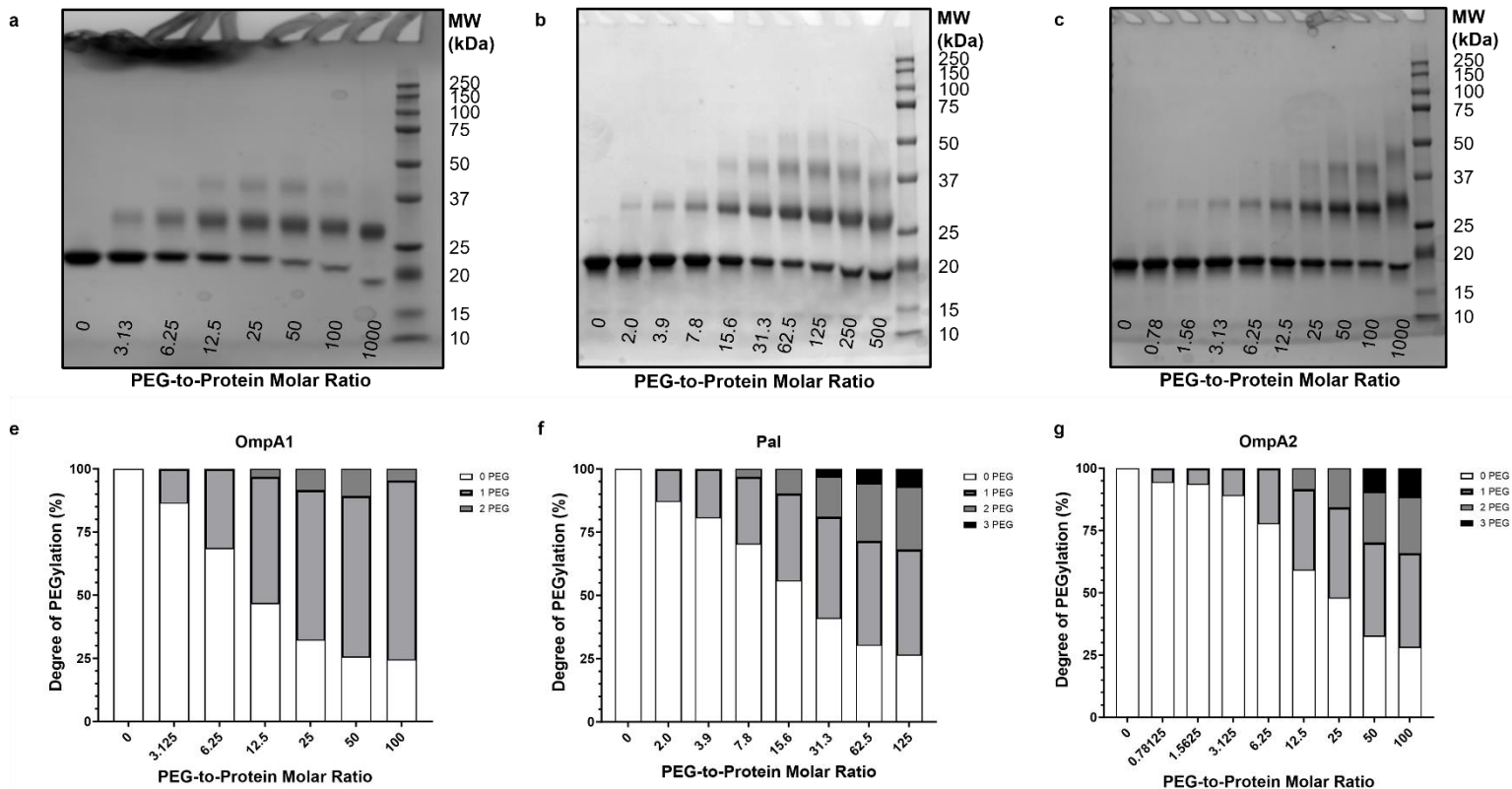


Fig S4: Optimization of Protein PEGylation. Aliquots of 1 mg/mL protein were reacted with the indicated molar ratio of 3.4 kDa SH-PEG-NHS linker. SDS-PAGE with Coomassie stain of PEGylated OmpA1 (**a**), Pal (**b**), and OmpA2 (**c**). ImageJ gel densitometric analysis of PEGylated OmpA1 (**d**), Pal (**e**), and OmpA2 (**f**). Expected molecular weights of non-PEGylated proteins: OmpA1 = 20.2 kDa, Pal = 17.5 kDa, OmpA2 = 16.3 kDa. Graphs made with GraphPad Prism.

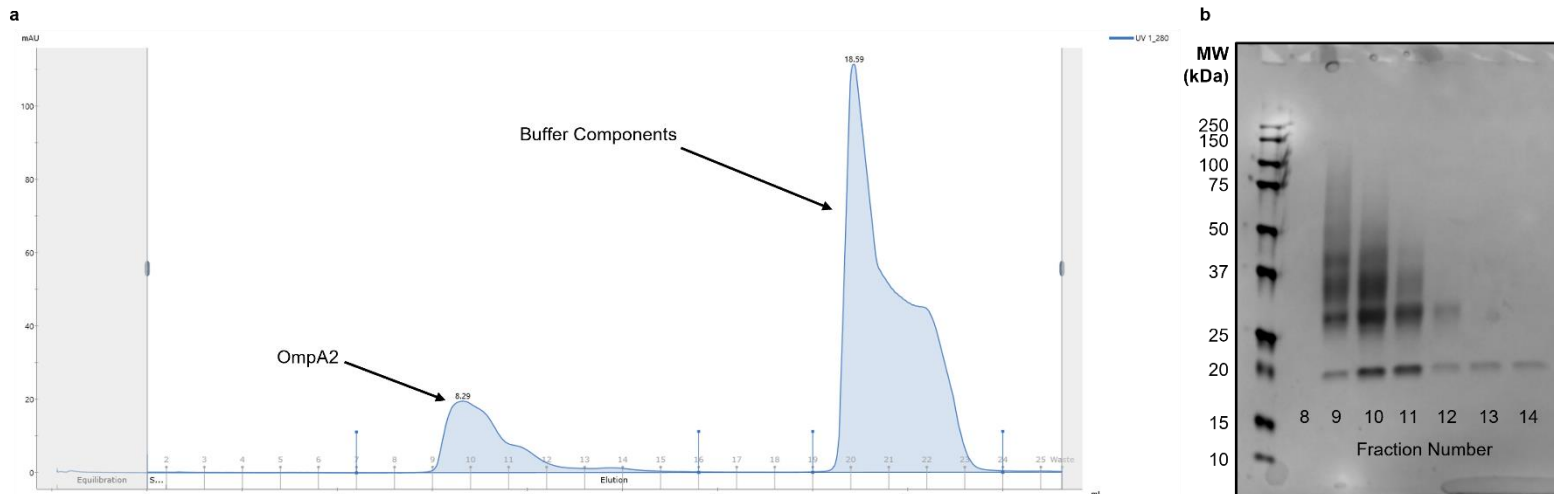


Fig S5: Size Exclusion Liquid Chromatography of PEGylated Proteins. PEGylated proteins were applied to a Cytiva Superdex 75 Increase 10/300 GL column connected to an ÄKTA pure protein purification system. **(a)** Representative chromatogram from purification of PEGylated OmpA2. The peak labelled “buffer components” is devoid of protein and is thought to contain the NHS leaving group, which absorbs strongly at 280 nm, as well as unreacted linker. **(b)** Representative SDS-PAGE with silver stain of chromatography fractions 8 through 14. The expected molecular weight of non-PEGylated OmpA2 is 16.3 kDa.

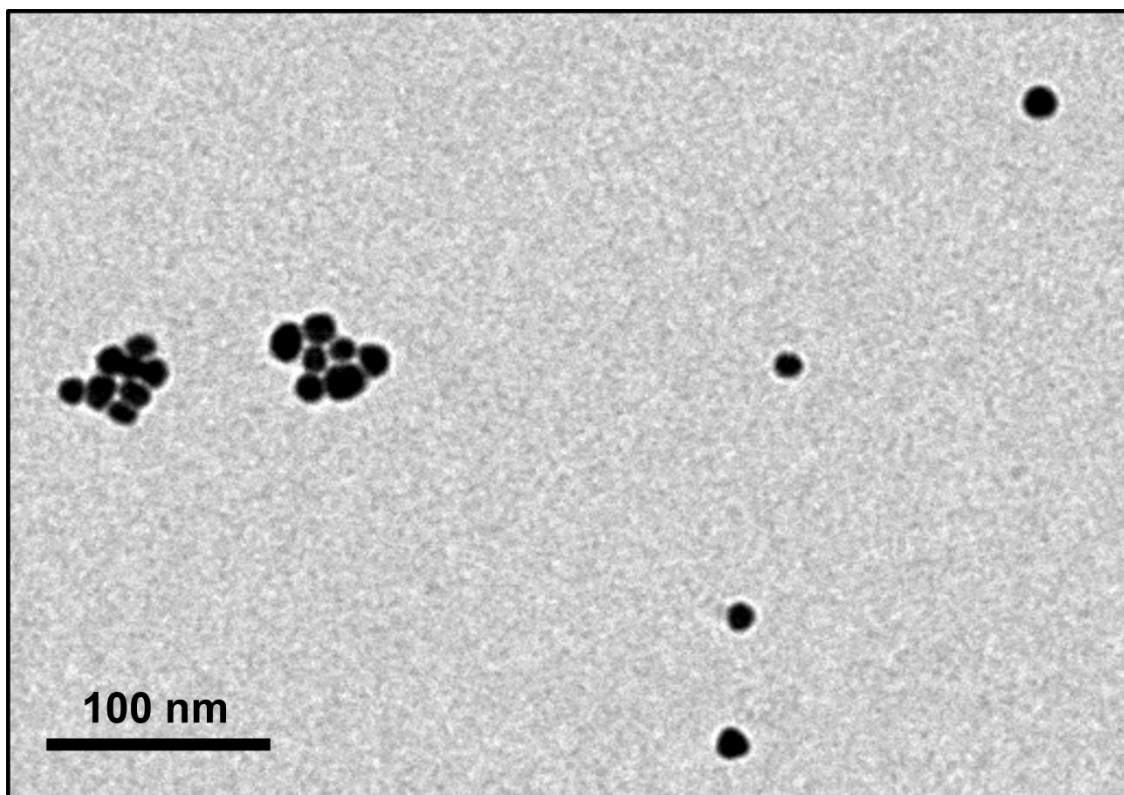


Fig S6: Transmission Electron Microscopy of Unmodified AuNPs. AuNPs were directly applied as a droplet to Formvar/Carbon 200 Mesh, Cu grids and imaged on a JEOL JEM-1400 transmission electron microscope.

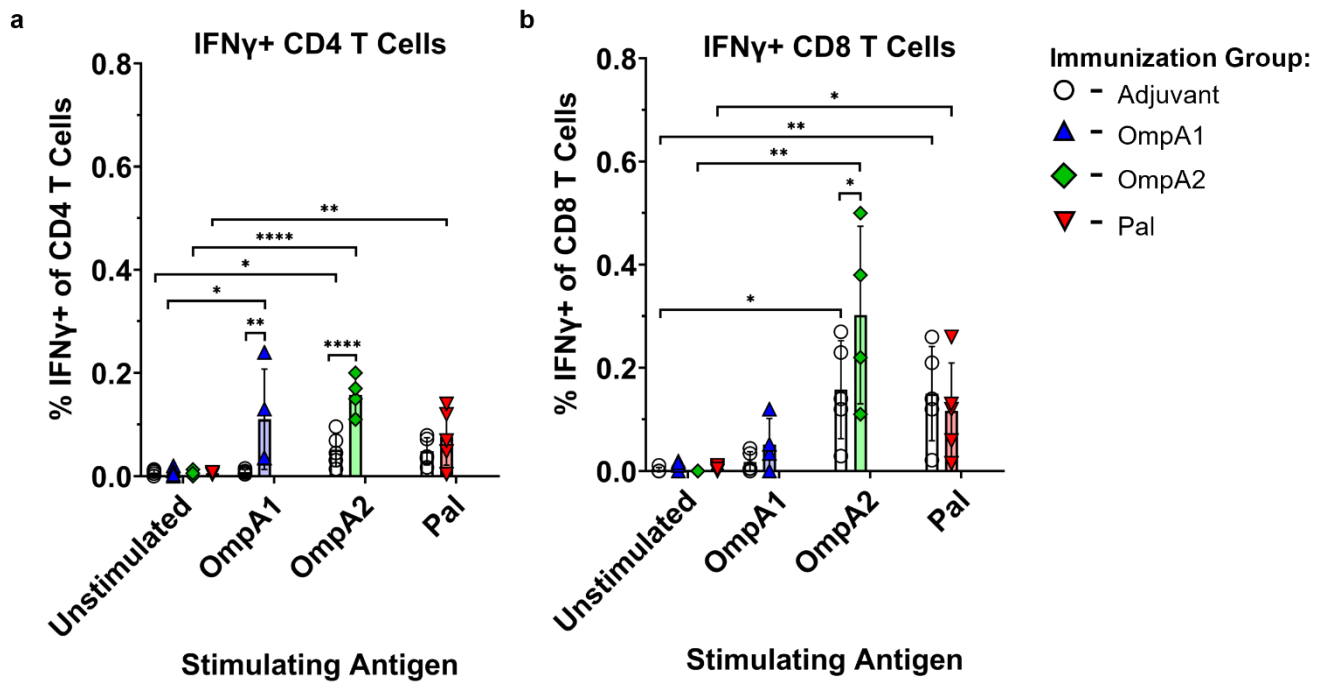


Fig S7: T Cell Recall Intracellular IFN γ Staining. Splenocytes from immunized animals were stimulated with the indicated recombinant protein for 24 h, stained with fluorescent antibodies, and analyzed via flow cytometry. Intracellular IFN γ staining of CD4 (**A**) and CD8 (**B**) T cells. Groups were compared via matched-pairs two-way ANOVAs with Fisher's LSD tests. (*) $p < 0.05$, (**) $p < 0.01$, (****) $p < 0.0001$. Graphs made in GraphPad Prism.

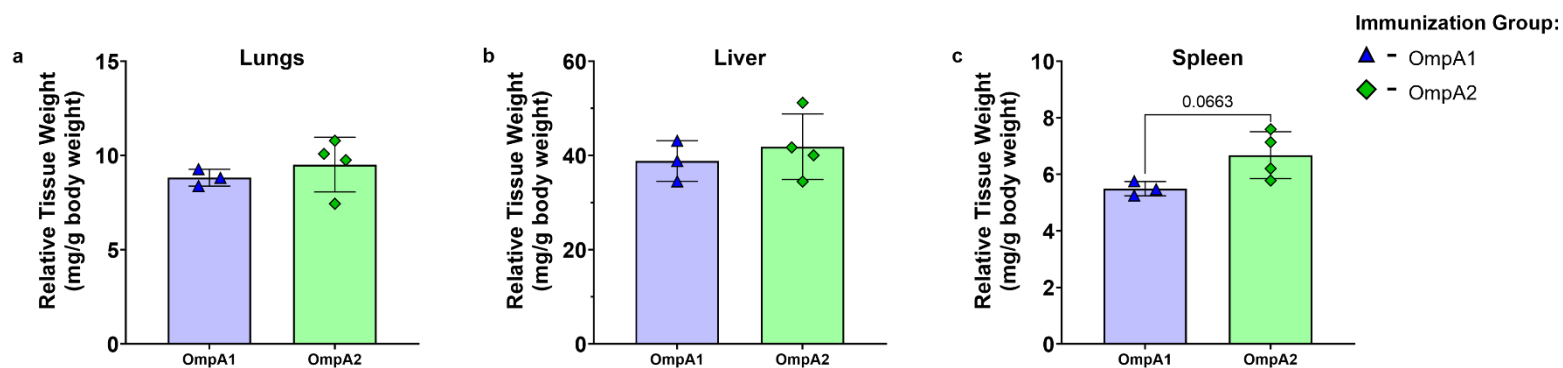


Fig S8: Tissue Weights Post-Infection. Lungs (A), livers (B), and spleens (C) were collected at the challenge study endpoint and weighed. Tissue weights were normalized to body weight at time of collection. Groups were compared by unpaired, two-tailed Student's t-tests.

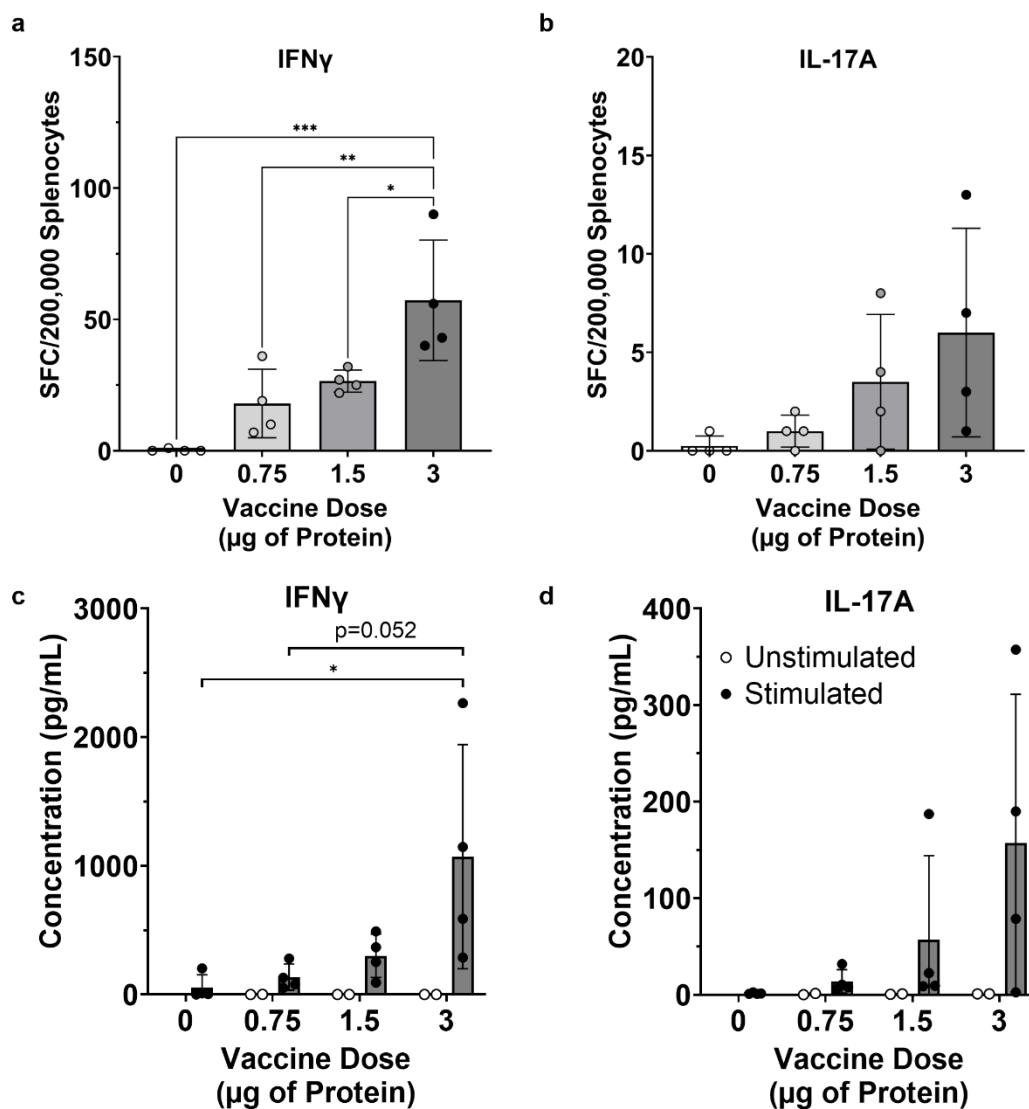


Fig S9: AuNP-OmpA2 Dosing Study T Cell Recall. Mice were immunized with either 0.75, 1.5, or 3 μ g/dose of OmpA2 conjugated to AuNPs and adjuvanted with Vaccigrade CpG ODN 2395. At the endpoint of the study (d38), spleens were collected, processed, and stimulated with 10 μ g/mL recombinant OmpA2 or vehicle control. IFN γ (**a**) and IL-17A (**b**) ELISpots. SFC = spot forming cells. Supernatants collected from stimulated cells were collected and probed for IFN γ (**c**) and IL-17A (**d**) using a LEGENDplex Mouse Th Cytokine Panel (12-plex). Groups were compared using one-way ANOVAs with Tukey post hoc. (*) p < 0.05, (**) p < 0.01, (***) p < 0.001.

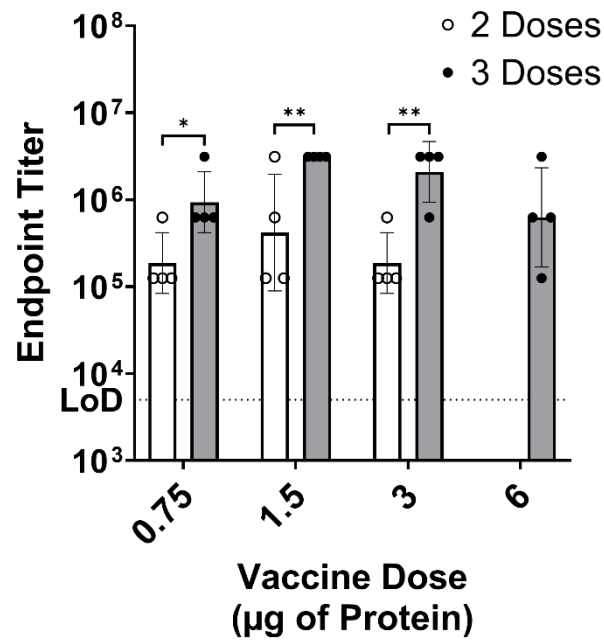


Fig S10: AuNP-OmpA2 Dosing Study Antigen-Specific IgG. C57BL/6 mice were immunized with either 0.75, 1.5, or 3 µg/dose of OmpA2 conjugated to AuNPs and adjuvanted with Vaccigrade CpG ODN 2395. Blood was collected one week after the second immunization (d21) and 10 days after the third immunization (d38) was probed for total IgG. Total IgG ELISAs were performed using serial diluted serum and using recombinant OmpA2 as the coating antigen. For comparison, endpoint titers from the high dose vaccination study (6 µg/dose; d42) were also included in the graph. Log transformed endpoint titers were compared using a matched pairs two-way ANOVA with Šidák correction. (*) $p < 0.05$, (**) $p < 0.01$.

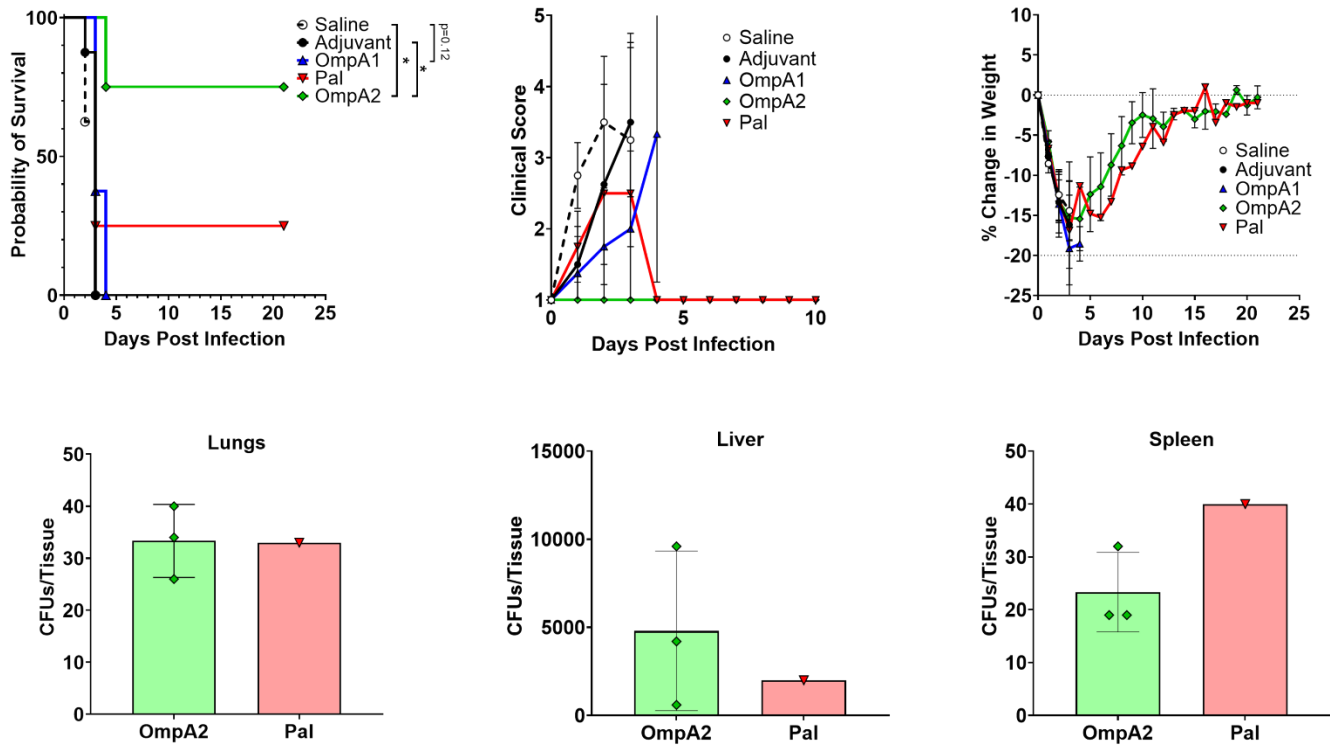


Fig S11: High Dose Vaccination and Challenge Study. C57BL/6 mice were immunized with a high dose of the indicated vaccine containing approximately 6 $\mu\text{g}/\text{dose}$ of protein. Three weeks after the third immunization, animals were challenged intranasally with $5.0 \times \text{LD}_{50}$ of *Bpm* K96243. **(a)** Survival is reported as a Kaplan-Meier curve. **(b)** Highest clinical score recorded daily up to 10 dpi. Clinical scores were reported as follows: 1 = active and healthy appearance; 2 = mild lethargy; 3 = ruffled fur, hunched posture, and mild lethargy; 4 = ruffled fur, hunched posture, limited mobility; 5 = moribund. **(c)** Percent change in weight from the day of infection. Lungs **(d)**, livers **(e)**, and spleens **(f)** from animals that survived to 21 dpi were homogenized, serially diluted, and plated to assess organ colonization. Survival curves were compared to the saline and adjuvant control groups using log-rank tests and adjusted p values were calculated using the Bonferroni method. (*) p < 0.05.

Primer	Sequence
BPSL0999 Forward	tgcacccatcatcatcatcatGCAACCCAGCAAGGCACC
BPSL0999 Reverse	tggtgggtgggtggctcgagTTACTGCGCCGCTTGCGG
BPSL2522 Forward	tgcacccatcatcatcatcatGTTGCTCCGGCCATCACG
BPSL2522 Reverse	tggtgggtgggtggctcgagTTACTGCGCCGGAACGGT
BPSL2765 Forward	tgcacccatcatcatcatcatAAGTCGGGCGTGAAGCTC
BPSL2765 Reverse	tggtgggtgggtggctcgagTTACTGTTGATAGACGAGGTCCG

Table S1: List of Primers.

OmpA1 (BPSL0999)		
Peptide	m/z	z
GAAIGAGVGLVGGVTGYNWQAIK	744.0708	3
NKLAPSAQQTGTQVTEQPDGSLK	800.0785	3
NKLAPSAQQTGTQVTEQPDGSLK	600.3107	4
LAPSAQQTGTQVTEQPDGSLK	1078.5451	2
LAPSAQQTGTQVTEQPDGSLK	719.3658	3
LAPSAQQTGTQVTEQPDGSLK	539.7762	4
AQSVVNALAQR	578.8253	2
GVAANRLSAQGMGASNPIADNATEAGR	867.0929	3
LSAQGMGASNPIADNATEAGR	1015.9816	2
LSAQGMGASNPIADNATEAGR	677.6568	3
LSAQGMGASNPIADNATEAGRAQNR	834.0701	3
LSAQGMGASNPIADNATEAGRAQNR	625.8044	4
RVEIYLR	474.7849	2
VEIYLR	396.7343	2
VEIYLRAPQAAQ	679.875	2
VEIYLRAPQAAQ	453.5857	3
AQSVVNALAQR(heavy)	583.8294	2
OmpA2 (BPSL2522)		
Peptide	m/z	z
IDEIAAK	421.5619	2
ITYQADTLFDLFDK	788.88166	2
QLIAC LAPDR	578.81081	2
EKPVALGHDEASWAQNR	954.4716	2
SYLVSKGVPANK	631.85863	2
VEVEVVGTQEVQK	726.4006	2
VEVEVVGTQEVQK	484.6012	3
RVEVEVVGTQEVQK	533.96062	3
RVEVEVVGTQEVQKTTVPAQ	733.06666	3
VEVEVVGTQEVQK	722.38916	2

Table S2: List of Targeted Peptides Included in the PRM Assay with Corresponding Precursor m/z Values and Charge States.