Supplementary Material and Methods:

Recombinant glycoprotein expression. Previous work by Iwashkiw *et al.* characterized the general *O*-glycosylation system in *A. baumannii* ATCC 17978 including the identification of 7 novel membrane-associated glycoproteins [1]. One of the glycoproteins, A1S_1193, was previously cloned with a C-terminal Deca-Histidine tag into pEXT20 and the L-arabinose inducible *E. coli/Acinetobacter* shuttle vector pEC [2]. In this work A1S_1193His was subcloned into *E. coli/Acinetobacter* shuttle vector pBAVMCS at *Bam*HI and *Sal*I where the protein is constitutively expressed (New England Biolabs, Whitby, ON, Canada). A Qiagen plasmid mini-preparation kit was used to purify the plasmid DNA encoding the Histidine tagged A1S_1193. The purified plasmid DNA was then electroporated into electro-competent *Acinetobacter* cells and plated onto LB agar plates containing 50ug/mL Kanamycin to select for the maintenance of the plasmid. Expression of this recombinant protein in *Acinetobacter* was assessed by Western-blot analysis using polyclonal anti-Histidine sera (1:4000) (Rockland Immunochemicals, Gilbertsville, PA). The nitrocellulose membrane was then probed with an IRDye conjugated anti-rabbit antibody and a Odyssey infrared imaging system (LI-COR Biosciences, Lincoln, NE) was used to visualize the blot. Modification of the glycoprotein was observed through Western blot analysis as an increase in molecular weight relative to the unglycosylated control produced in the ATCC 17978 Δ*pglL* strain. [1].

Purification and mass spectrometric analysis of the tagged glycoprotein **A1S_1193.** A1S_1193 was purified by Nickel-affinity chromatography as previously described [2]. Briefly, membranes were prepared from overnight cultures by suspending cells in ice-cold PBS containing Complete EDTA-free protease inhibitor cocktail (Roche) and lysed using 3 rounds of disruption at 30Kpsi using a cell disruptor (Constant system ltd.). Unbroken cells and insoluble cell debris were

clarified at 10,000x g for 10 minutes, 4°C and the resulting supernatant was subjected to ultracentrifugation, 100,000x g for 60 minutes, 4°C, yielding a pellet of bacterial membrane. The resulting pellet was solubilized overnight at 4°C in 1% Triton X-100 in PBS by tumbling. The detergent concentration was decreased to 0.5% by dilution with PBS and insoluble membrane components removed by ultracentrifugation at 100,000x g, 60 minutes, 4°C. Solubilized membrane protein extracts were purified using Ni-sepharose media on a ATKA explorer, previously equilibrated with buffer A (20mM Tris-HCl pH 8.0, 300mM NaCl, 0.5% Triton X-100) with 10mM Imidazole. Extracts were washed with 5mL of buffer A with 10mM Imidazole at a flow rate of 1mL/min to remove unbound proteins. Higher stringency washes were done with buffer A with 30mM Imidazole at 1mL/min and elution was performed at 0.5mL/min with buffer A with 250mM Imidazole in 1mL fractions.

Periodic Acid Schiff (PAS) staining of total membrane extracts resolved by SDS-PAGE. Total membrane extracts from *A. baumannii* were isolated as described above. The protein content of the membrane extracts was quantified by a Bradford assay (Bio-rad) and resolved on a 12% SDS-PAGE gel. The general carbohydrate PAS staining protocol has been previously described [3]. Briefly, 0.7% periodic acid in 5% acetic acid oxidizes *cis*-diol sugars, sensitizing them for staining with Schiff reagent (Sigma).

Complement mediated killing assay for A. baumannii. This assay for capsular polysaccharide mediated resistance to complement-mediated killing was performed similarly as previously described [4]. Overnight cultures of *A. baumannii* were re-inoculated into LB media and grown at 37^{o}C 200RMP for 4 hours. Aliquots were pelleted at 5000*g* for 10 minutes, washed in sterile PBS, pelleted again and re-suspended in 100% horse serum (Gibco) at 1 OD/mL. Serial 10-fold dilutions in 180uL of serum were performed in a 96-well plate. 10uL of each dilution series was

plated onto LB agar immediately after re-suspension in serum and 3 hours post incubation at 37^{o}C. Plates were incubated overnight at 37^{o}C to allow growth of surviving bacteria.

References:

1. Iwashkiw JA, Seper A, Weber BS, Scott NE, Vinogradov E, et al. (2012) Identification of a general O-linked protein glycosylation system in Acinetobacter baumannii and its role in virulence and biofilm formation. PLoS Pathog 8: e1002758.
2. Madsen JA, Ko BJ, Xu H, Iwashkiw JA, Robotham SA, et al. (2013) Concurrent automated sequencing of the glycan and peptide portions of O-linked glycopeptide anions by ultraviolet photodissociation mass spectrometry. Anal Chem 85: 9253-9261.
3. Cagatay TI, Hickford JG (2008) Glycosylation of type-IV fimbriae of Dichelobacter nodosus. Vet Microbiol 126: 160-167.
4. Lees-Miller RG, Iwashkiw JA, Scott NE, Seper A, Vinogradov E, et al. (2013) A common pathway for O-linked protein-glycosylation and synthesis of capsule in Acinetobacter baumannii. Mol Microbiol 89: 816-830.