Purification of L,D-transpeptidases from *K. pneumoniae*

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INTRODUCTION

Since the discovery of penicillin in 1928, antibiotics have been used worldwide to treat illness resulting from colonization of pathogenic bacteria. The most widely prescribed penicillin-based antibiotics are β-lactams (**Figure 1A**).[1] β-lactams work by interfering with cell-wall synthesis by inhibiting penicillin-binding proteins (PBPs). Numerous resistance mechanisms oriented toward β-lactams have emerged over the years. The most common resistance mechanism is the expression of β-lactamases, enzymes that are able to hydrolyze the β-lactam ring, which renders the antibiotic useless.[2] These resistance mechanisms pose a massive threat to the effectiveness of β-lactams. Even with the development of carbapenems, β-lactams designed to bypass β-lactamases, carbapenem-resistant Enterobacteriaceae (CRE) such as *Klebsiella pneumoniae* have arisen. The emergence of CRE highlights the need for a better understanding of the various mechanisms of resistance towards β-lactam antibiotics.

In addition to β-lactamases, many species of Enterobacteriaceae also express L,D-transpeptidases (Ldts) which are structurally and functionally different enough from D,D-transpeptidases (PBPs) to not be recognized by β-lactams.[3] Ldts form a different crosslink in the peptidoglycan layer of the cell-wall of bacteria than PBPs (**Figure 1B**). While Ldts do not normally serve as the primary pathway of cell-wall biosynthesis, it is hypothesized that while under pressure from a β-lactams, Ldts can assume a primary role and allow the bacteria to survive. CRE are an ever-growing problem in the umbrella of antibiotic resistance, as they are a large source of hospital-acquired infections and are often life-threatening.[4] Because of this, it is important to determine which, if any, β-lactam antibiotics are able to retain activity against the Ldts expressed by CRE organisms.

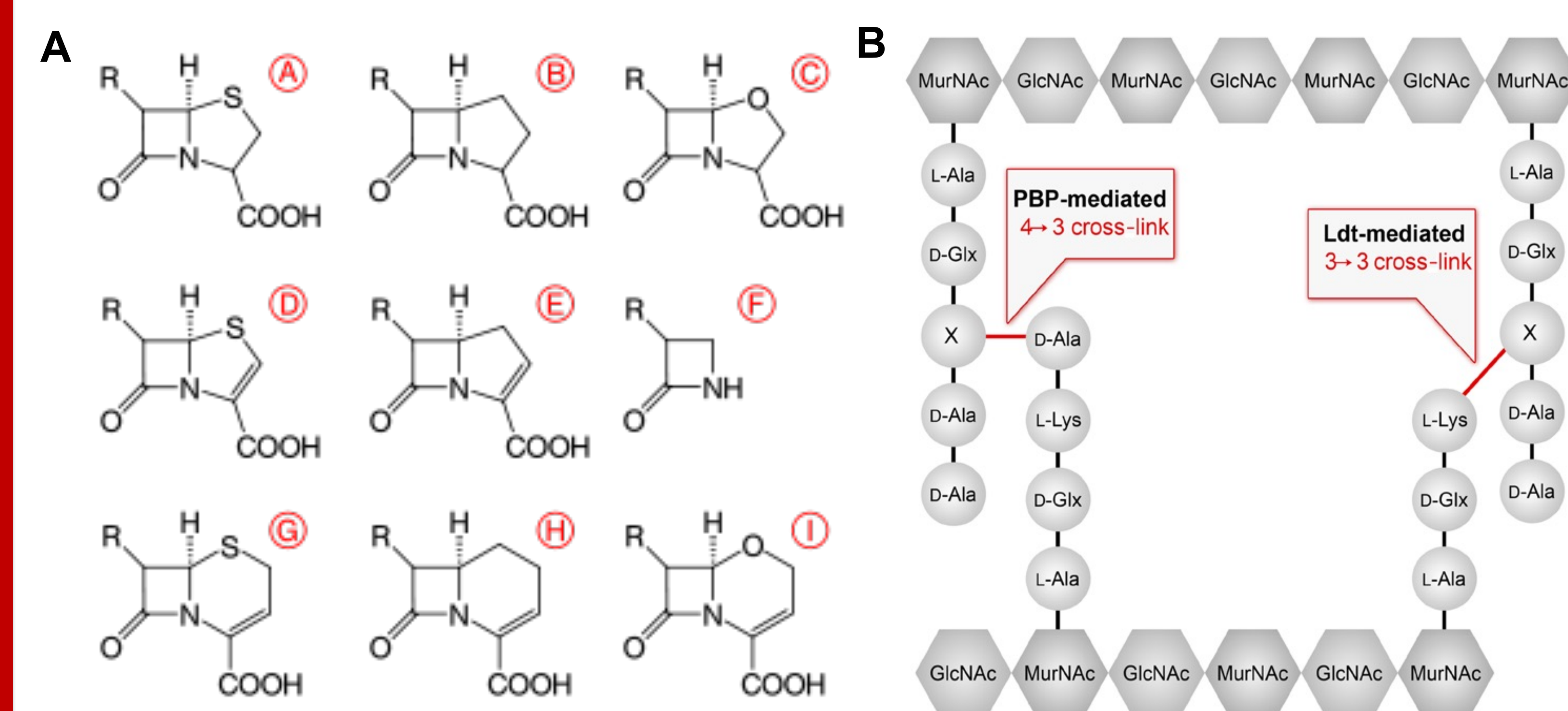

Figure 1. Structures of β-lactams and cell-wall cross-links. (A) Classes of β-lactams. A) penam, B) carbapenem, C) oxapenam, D) penem, E) carbapenam, F) monobactam, G) cephem, H) carbacephem, and I) oxacephem. (B) Structural differences between peptidoglycan cross-links synthesized by PBPs versus Ldts.[3]

REFERENCES & FUNDING

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1) Bush K, Bradford PA. *Cold Spring Harb Perspect Med* (2016) **6**, a025247.
2) Nordmann P. *Clin Infect Dis* (1998) **27**, S100.
3) Pidgeon SE, *et al. ACS Chem Biol* (2019) **14**, 2185.
4) Suay-Garcia B, Perez-Gracia MT. *Antibiotics* (2019) **8**, 122.

METHODS & RESEARCH DESIGN

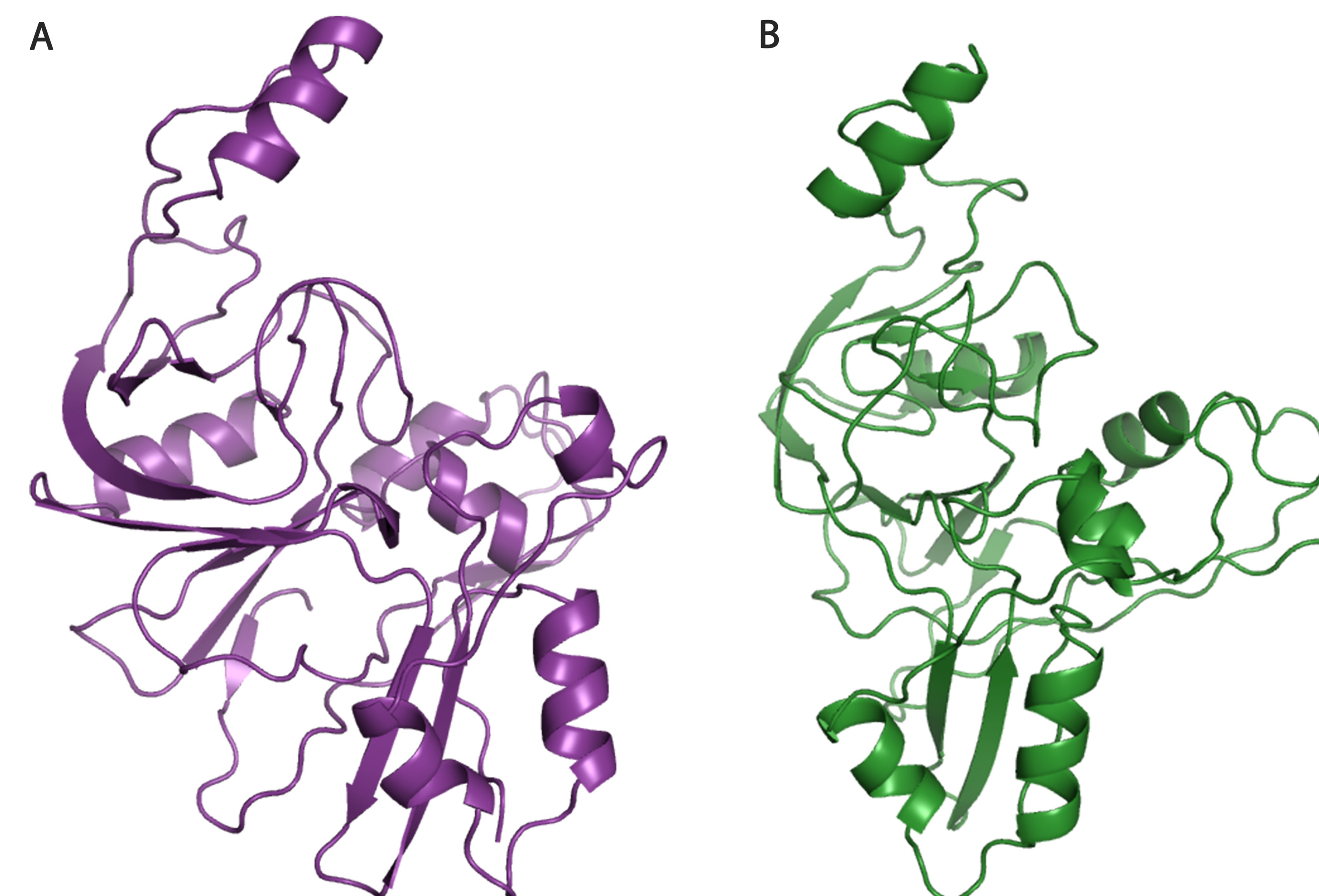

Figure 2. Homology models of *K. pneumoniae* Ldts. *K. pneumoniae* (A) LdtB and (B) YnhG were modelled using MODELLER from the HHpred server using published structures of L,D-transpeptidases (PDB IDs: 4LZH, 4A1K, 3ZG4, 6RLG).

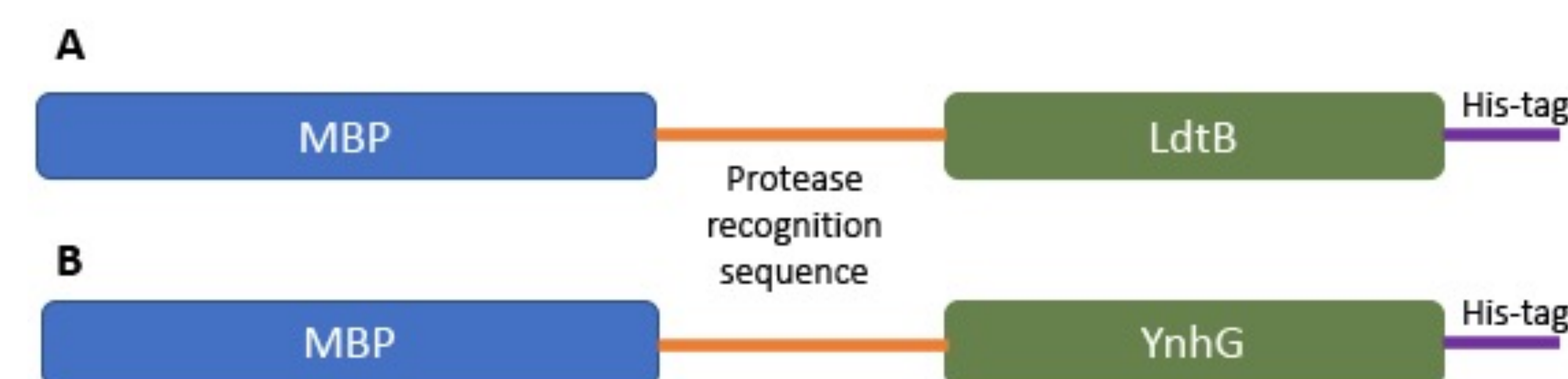

Figure 3. Design of MBP-Ldt fusion protein constructs. Maltose-binding protein (MBP) was fused to the respective Ldt to facilitate expression and solubility; (A) LdtB and (B) YnhG from *K. pneumoniae.* A linker containing a TEV protease recognition site and a hexahistidine tag were also included.

Purification Protocol.

Step 1
- Overexpression of START fusion in *E. coli,* followed by lysis of cells and isolation of soluble fraction
- Nickel-affinity chromatography of lysate

Step 2
- Buffer exchange of Ni-column elution fractions into a low salt buffer
- Ion-exchange chromatography

Step 3
- Concentrate ion-exchange fractions
- Size-exclusion chromatography

RESULTS

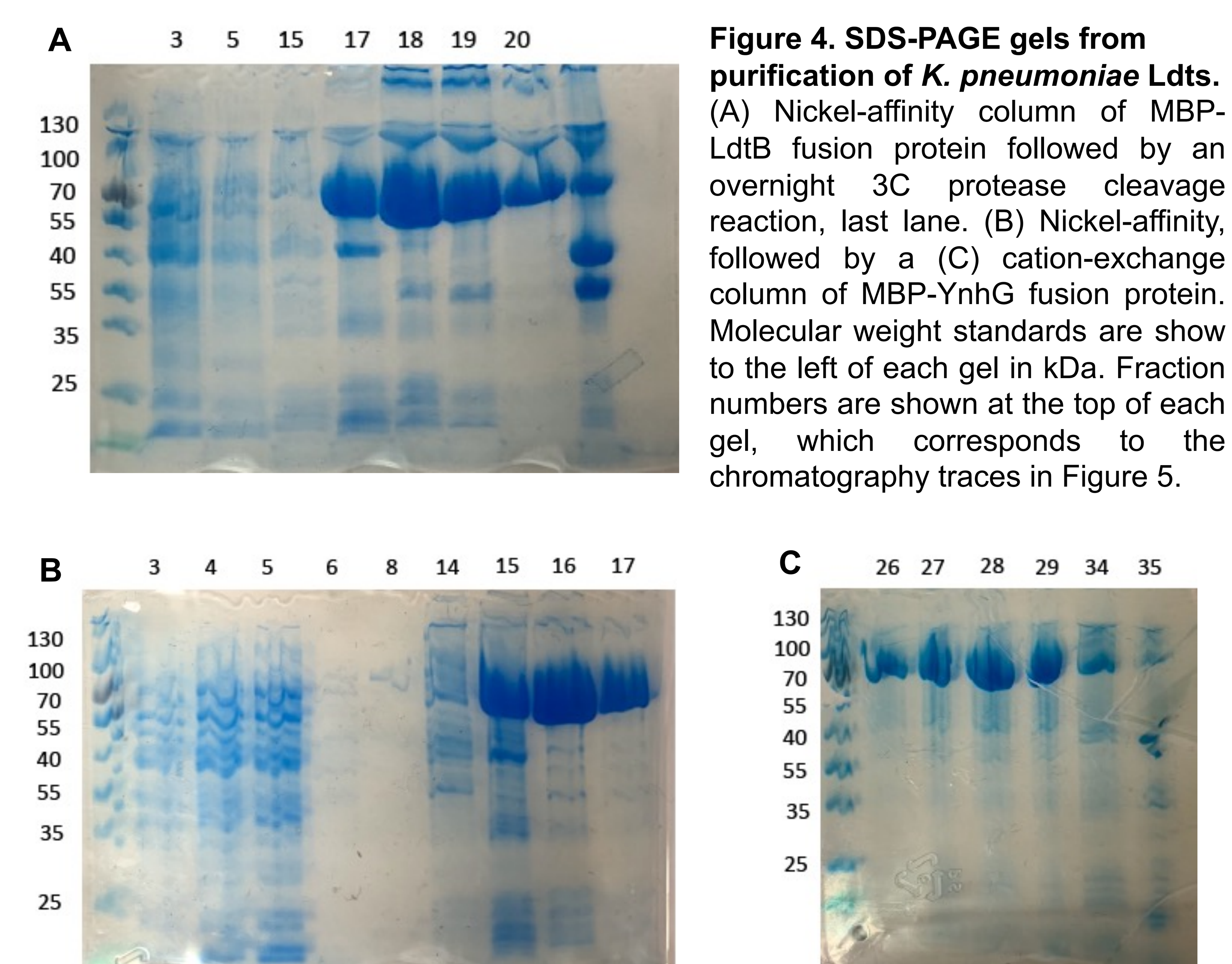

Figure 4. SDS-PAGE gels from purification of *K. pneumoniae* Ldts. (A) Nickel-affinity column of MBP-LdtB fusion protein followed by an overnight 3C protease cleavage reaction, last lane. (B) Nickel-affinity, followed by a (C) cation-exchange column of MBP-YnhG fusion protein. Molecular weight standards are show to the left of each gel in kDa. Fraction numbers are shown at the top of each gel, which corresponds to the chromatography traces in Figure 5.

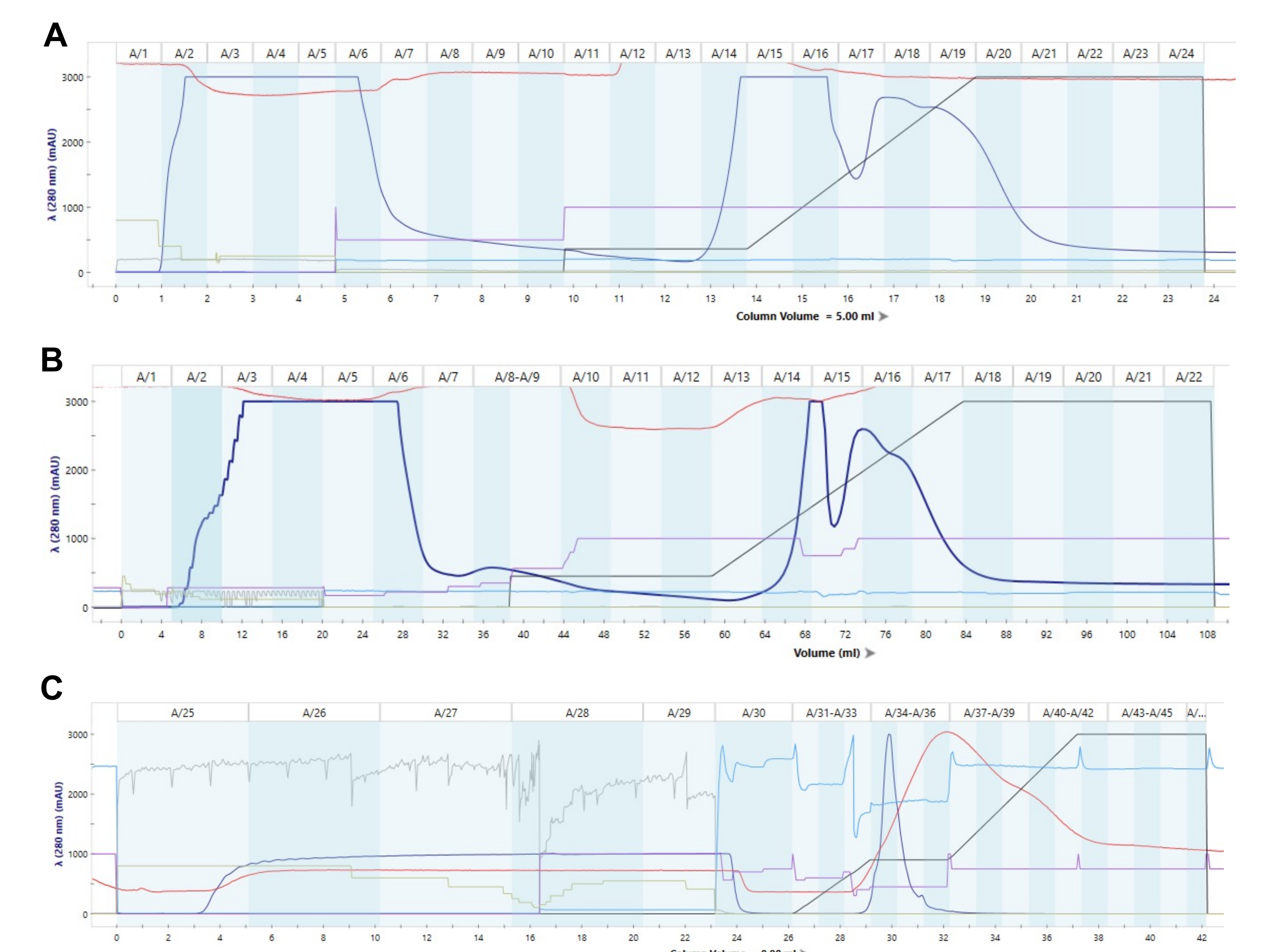

Figure 5. Chromatography traces for the purification of *K. pneumoniae* Ldts. (A) Nickel-affinity column for MBP-LdtB fusion. (B) Nickel-affinity and (B) cation-exchange column of MBP-YnhG. The dark blue line shows the absorbance at a wavelength of 280 nm. The red trace shows the conductivity of the sample. Samples from fractions that showed absorbance were ran on SDS-PAGE, shown in Figure 4.