299c Strategies for Expanding the Repertoire of Proteins That Can Be Displayed on the Outer Surface of *E. Coli*

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Anchoring heterologous proteins on the cell surface of different host organisms is a powerful methodology with a broad range of biotechnological applications including bacterial vaccines, high-throughput screening of peptide, antibody, and enzyme libraries, whole cell sorbents, recombinant biocatalysts, and cell-based diagnostics. In addition, surface localization of proteins is of fundamental importance to biology and human health as the mechanisms for protein transport and membrane integration in bacteria are not completely understood and oftentimes play a key role in pathogenesis.

Cytolysin A (ClyA) is a pore-forming toxin of *Escherichia coli*, *Salmonella typhi*, and *Shigella flexneri*. Recent data demonstrates that ClyA is integrated in the outer membrane where it forms an octameric assembly. However, very little is known about its mechanism of transport across the bacterial inner membrane. In addition, whether ClyA might serve as a suitable membrane anchor for cell surface display has not been demonstrated. We have generated chimeras between ClyA and a number of heterologous proteins (e.g. GFP, b-lactamase, organophosphohydrolase and numerous scFv sequences) in order to test the utility of the ClyA secretion mechanism for anchoring proteins to the outer surface of E. coli. Our results demonstrate that ClyA is a promising and unique anchoring motif, not only for tethering to the outer surface of bacteria but also for display on the surface of outer membrane vesicles (OMV) released by bacteria. In addition, using these chimeric proteins as genetic reporters, we demonstrate that ClyA is translocated via a Sec-independent mechanism. Thus, an inherent benefit of this engineered ClyA system is that it bypasses many of the inherent drawbacks associated with Sec-mediated surface display (e.g. jamming of the Sec pore, limited folding environment in the periplasm).