AI-Designed Protein Inhibitors can block heme uptake and inhibit growth of Pathogenic *E. coli*

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Iron is an essential nutrient for most bacteria and is often a limiting nutrient during infection due to the host sequestering free iron as part of the innate immune response. Pathogenic *E. coli* and *Shigella sp*. produce the outer membrane transporter ChuA, which binds host hemoglobin and extracts its heme cofactor, before importing heme into the cell. Heme extraction by ChuA is a dynamic process, with the transporter capable of rapidly extracting heme from hemoglobin in the absence of an exogenous energy source, and without forming a stable ChuA-hemoglobin complex. In this study, we solved the crystal structure of ChuA in complex with heme extracted from human haemoglobin, identifying residues required for coordination of heme. In addition, we modelled the ChuA-haemoglobin complex using AlphaFold and identified a hydrophobic haemoglobin binding region in the extracellular binding loops of ChuA, which is contiguous with the heme binding region. Based on these data, we have developed a putative mechanism defining initial ChuA-haemoglobin interaction and subsequent heme extraction. To test this model, we generated a panel of ChuA mutants in key residues from this region and validated their importance for binding haemoglobin and heme extraction using growth assays, and further purified them and characterised their ability to bind haemoglobin. Using RFDiffusion, we twice directed the design of 10,000 de novo proteins to bind at the ChuA:haemoglobin binding interface, and selected and tested 96 of the best candidates for their ability to bind ChuA and block growth on haemoglobin. We identified several binders that inhibit *E. coli* growth at low nM concentrations, without further optimisation. Finally, we determine the cryoEM structure of a subset of these binders, alone and in complex with ChuA, demonstrating that they closely match the computational design. As such this work demonstrates the utility of AI-designed de novo proteins as potent inhibitors of pathogenic bacterial outer membrane transporters, and ultimately highlights the power of AI in the design of next-generation therapeutics.