Iron is an essential nutrient for most bacteria. However, it is highly insoluble under aerobic conditions making it a limiting factor for bacterial growth [1]. Iron limitation is a major barrier for pathogenic microbes due to host nutritional immunity, where iron is sequestered by human iron-containing proteins such as transferrin or in heme bound by proteins such as haemoglobin [2]. This sequestration makes iron inaccessible to bacteria, and in order to infect the host, pathogenic bacteria must steal it from iron-containing proteins, often using TonB-dependent transporters (TBDTs) present in the Gram-negative bacterial outer membrane [3]. The TBDT ChuA from pathogenic *Escherichia coli* binds host haemoglobin in order to scavenge heme. Once liberated, heme is shuttled through ChuA into the periplasm and then actively transported into the cytoplasm for further processing [4]. ChuA has been shown to acquire heme from haemoglobin; however, whether ChuA is selective for haemoglobin over that of free heme was uncertain. In this study, we solved the structure of ChuA in complex with heme extracted from human haemoglobin to a resolution of 3.1Å, identifying residues required for coordination of heme. In addition, we modelled the ChuA-haemoglobin complex using AlphaFold2 [5], 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. In addition, we expressed and purified these mutants and further characterised their ability to bind haemoglobin. Identification and characterisation of key residues required for binding in ChuA highlights their potential for the development of inhibitory compounds or nanobodies.