The bacterium *Clostridium perfringens* causes severe, sometimes lethal gastrointestinal disorders in domesticated mammals and humans, with type F strains producing an enterotoxin (CpE) that causes the 3rd most common foodborne illness in the US. CpE breaks down the gut barrier by recognizing then binding surface-exposed receptors, triggering dissociation of their multi-protein complexes. The receptors, a 27-member family of integral membrane proteins called claudins, are the structural and functional backbone of tight junctions. CpE dissociation of claudins also disrupts tight junctions, which direct epithelial cell/cell adhesion and are barriers to paracellular molecular transport. While it was known that claudin recognition is encoded in CpE’s C-terminal domain (cCpE) and that only select claudins present in mammalian genomes bind CpE well, the molecular and structural bases for claudin targeting by CpE was undetermined. We clarified CpE’s nuanced detection of claudins by determining the structure of human claudin-4 in complex with cCpE. The structure reveals that cCpE targets a 12-amino acid motif on claudin-4 and that Leu151 penetrates a cCpE groove. Establishing the binding affinities, kinetics, and half-lives between divergent and mutant subtypes and CpE showed that Leu151 imparts high-affinity binding by decreasing the dissociation rates and increasing the half-lives of complexes. Quantifying claudin/CpE complex life spans permitted novel classification of receptor/non-receptor subtypes, uncovering that the primary CpE receptors differ in mice and humans. Sequence analysis further verified that Leu151 and the 12-residue motif are discriminating features due to conservation in receptive but not non-receptive subtypes. *In vivo*, however, CpE-induced cytotoxicity of cells expressing both classes shows that CpE kills equivalently, indicating CpE broadly recognizes all claudins. These findings establish the structural and biophysical basis of CpE’s micro- and nano-recognition of claudins with varied receptive capacities and helps elucidate CpE’s targeting mechanism during pathogenicity of *Clostridium perfringens* in mammalian gut. This knowledge provides a framework for new strategies to treat CpE-type gastrointestinal illnesses in domesticated mammals and humans and for designing new CpE-based therapeutics for tuning permeability of the blood-brain barrier and for detecting cancer.