Most globular proteins are thought to be only marginally stable. The main stabilising forces for a protein scaffold are considered to be the numerous weak interactions forming between the side chains and the backbone of a folded polypeptide chain. Despite the diversity of amino acid side chains, only cysteines were known to form covalent (disulfide) bonds that stabilise protein scaffolds.

The discovery of isopeptide bonds in the surface protein of the Gram-positive bacterium Streptococcus pyogenes changed this simplistic view (Kang et al. 2007). Isopeptide bonds are formed auto-catalytically between lysine and asparagine or an aspartate residues that are brought together in a hydrophobic environment during protein folding. More recently, new intramolecular crosslinks formed between threonine and glutamine side chains (ester bonds) were discovered in the surface protein Cpe0147 of another Gram-positive bacterium, Clostridium perfringens (Kwon et al. 2014).

Similarly to the isopeptide bonds, ester crosslink formation appears to be an auto-catalytic reaction, in this case with a mechanism analogous to that of a serine protease utilising a catalytic triad of threonine, histidine, and aspartate residues. However, unlike the serine protease mechanism where a water molecule attacks and hydrolysates an acyl intermediate to regenerate the active site (and produce a cleaved peptide), the ester bond in the Cpe0147 is stable and does not react further.

We have begun to elucidate the full mechanism of ester bond formation in the Cpe0147 protein and to define the structural and chemical factors involved in this autocatalytic reaction. To do this, we are following ester bond formation via Nuclear Magnetic Resonance (NMR), and are probing the steric and chemical determinants of bond formation via site directed mutagenesis.


**Keywords:** Enzyme mechanism, Gram-positive bacteria, covalent bond