

**P017** Regulation of interaction between IRAK4 and MyD88 death domain by autophosphorylation.

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Insects and vertebrates have a powerful innate immune system that defends them against pathogens. The innate immune system in vertebrates is mediated through Toll-like receptor (TLR) signalling. Interleukin-1-receptor associated kinase (IRAK4), is a serine/threonine kinase that plays a key role in the early stages of TLR signalling. We have cloned and expressed human IRAK4 as a GST-fusion in *E. coli*. *In vitro* kinase activity assays showed that IRAK4 not only phosphorylated substrate, but autophosphorylated. At least ten autophosphorylation sites were mapped using mass spectrophotometry. They were found to be S8, T62, T111, T119, T208, T342, T345, S346, T352 and T365. Preliminary results suggest that autophosphorylation of full length IRAK4 is likely to be a sequential ordered reaction and not a random process, as demonstrated in the FGF-receptor-1 tyrosine kinase. The autophosphorylation sites within the IRAK4 death domain might play a functional role in regulating the binding and dissociation of MyD88 death domain. We have also reconstituted a stable complex of IRAK4 and MyD88 death domains, which is being characterised using analytical ultra centrifugation (AUC) and isothermal titration calorimetry (ITC).