MyD88 TIR domain higher-order assembly interactions revealed by microcrystal electron diffraction and serial femtosecond crystallography

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Myeloid differentiation primary response gene 88 (MyD88) and MyD88 adaptor-like/TIRAP (MAL) are Toll-like receptor (TLR) adaptors that signal to induce proinflammatory cytokine production. The TLR responds to a pathogen or endogenous molecules identified as 'dangerous' to our cells. This results in triggering the cytoplasmic/interleukin-1 receptor followed by the recruitment of adaptor proteins MAL and MyD88. We previously observed that the TIR domain of MAL (MAL^{TIR}) forms filaments in vitro and induces formation of crystalline higher-order assemblies of the MyD88 TIR domain ($MyD88^{TIR}$). These crystals are too small for conventional Xray crystallography but are ideally suited to structure determination by microcrystal electron diffraction (MicroED) and serial femtosecond crystallography (SFX). Here, we present MicroED and SFX structures of the $MyD88^{TIR}$ assembly, which reveals a two-stranded higherorder assembly arrangement of TIR domains analogous to that seen previously for MAL^{TIR}. Through mutagenesis studies we reveal the $MyD88^{TIR}$ assembly interfaces are critical for TLR4 signaling, in vivo, and provide evidence to show MAL promotes unidirectional assembly of $MyD88^{TIR}$. Together our results provide structural and mechanistic insight into TLR signal transduction and allows a direct comparison of the MicroED and SFX techniques on the same protein crystal [1].

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