

P046 Identification of the TLR3 signalling complex
Joshua N. Leonard (1), Janine Askins (1), Yan Wang (1), Rodolfo Ghirlando (2), Joseph Shiloach (3), Jessica Bell (4), David Margulies (5), David Davies (2) and David M. Segal (1)

National Institutes of Health, USA (1) NCI/EIB, (2) NIDDK/LMB, (3) NIDDK/Biotechnology Unit, (5) NIAID/LI/MBS; (4) Virginia Commonwealth University, Department of Biochemistry, USA

We have identified the mechanism by which Toll-like Receptor 3 (TLR3) interacts with its ligand, double-stranded RNA (dsRNA), to initiate signalling. Using purified human TLR3 ectodomain (TLR3ecd), we demonstrate that dsRNA binds specifically and reversibly to a site on the TLR3ecd. This binding was quantified using surface plasmon resonance, which revealed that the affinity is a function of both dsRNA length and the pH at which the interaction occurs. Interestingly, analytical ultracentrifugation and gel filtration analyses showed that TLR3ecd binds in pairs to dsRNA, even though the protein is monomeric in the absence of ligand. By using cell lines that express TLR3 both on the cell surface and in endosomes, we found that crosslinking of TLR3 on the surface (by antibodies) was sufficient to activate TLR3, but that binding and activation of TLR3 by dsRNA occurs only in the acidic milieu of endosomes. By using various sized dsRNA oligomers, we show that the minimal unit signal for activation is a 2:1 complex of TLR3 with dsRNA.