**MODULAR CONTROL OF INNATE IMMUNE SIGNALING USING SELF-ASSEMBLY OF IMMUNE SIGNALS**

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Vaccines play an increasingly important role in preventing and treating diseases ranging from infectious pathogens to cancer because these technologies harness the specificity of the immune system to clear disease without targeting the body's own cells. To realize these goals, new understanding of adjuvants – molecules added to vaccines to enhance function – is needed to support design of next-generation vaccines that elicit responses tailored for specific diseases. We recently reported a simple nanotechnology platform based on self-assembly of peptide antigen and a molecular toll-like receptor agonist (TLRa) to create modular vaccine designs (*ACS Nano* 2016, *ACS Nano* 2015). These structures – termed immune polyelectrolyte multilayers (iPEMs) – juxtapose antigen and TLRa at high densities, and offer 100% cargo loading since no carrier component is needed. This modularity also creates the possibility of rationally designing iPEMs that trigger multiple immune pathways with distinct control over the relative activation levels. In cancer, for example, activating multiple innate pathways has been linked to improved patient outcomes in human clinical trials. To exploit iPEMs in this manner, we designed iPEM architectures incorporating a conserved human cancer antigen (Trp2), and a range of molecularly-defined TLRa that spanned different TLRa classes and species (i.e., mouse and human): agonists for TLR3, TLR9, and TLR13. iPEMs were assembled from Trp2 and one, two, or three TLRas, or alternatively, using two different TLRas at varying compositions. To form carrier free capsules using these design schemes, Trp2 was appended with cationic amino acids, then adsorbed onto a sacrificial colloidal template, with alternating adsorption steps employing the specified TLRas (anionic). Centrifugation and wash steps were performed after each adsorption, then the template was dissolved using a chelator (EDTA) to form carrier-free capsules formed entirely from tumor peptide and each TLRa composition. All components were labeled to facilitate measurement of composition by fluorimetry and confocal microscopy. Using this approach, we discovered iPEMs could be assembled from any combination of Trp2 and the TLRas (**Fig. 1A**). Quantification revealed further confirmed iPEM capsules consisting of the corresponding peptide antigen and TLRa ligands (**Fig. 1B**). iPEMs incubated with primary dendritic cells isolated from BL6 mice revealed a high degree of colocalization of each iPEM component within cells. For example, iPEMs consisting of Trp2 and all three TLRas revealed that 90% of cells positive for at least one iPEM component were positive for all four components (**Fig. 1C**). Compared with equivalent free mixtures, iPEMs drove synergistic activation of DCs measured using flow cytometry for common surface activation makers (e.g., CD80, CD40, CD86) (**Fig. 1D**). Importantly, iPEMs also allowed modular control of TLR signaling, revealed using iPEMs built from Trp2 while varying the input ratio of TLR3a:TLR9a to control the final composition. iPEMs with a high TLR3a:TLR9a ratio triggered a high level of TLR3 signaling (**Fig. 1E**). As the ratio decreased, TLR3a signaling decreased, while TLR9a signaling increased. This rational control could contribute to more effective vaccines that use molecular adjuvant assembly to direct specific combinations and levels of multiple innate signaling pathways.

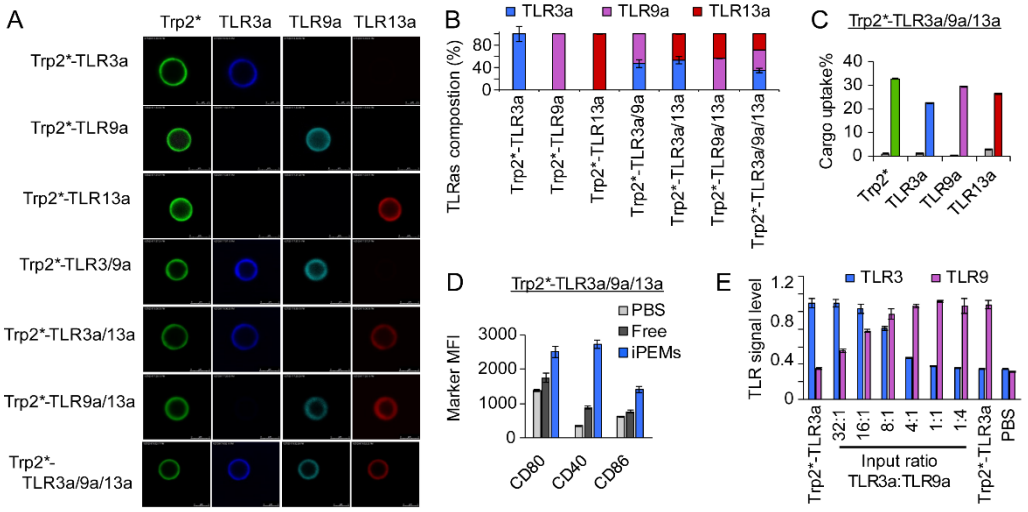


*Figure 1 – A) Modular assembly of Trp2 and combinations of TLRas in iPEMs. B) Quantification of TLRa loading. C) iPEMs deliver multiple signals to DCs, and D) drive synergistic responses relative to equivalent free signals. E) iPEMs can be prepared with distinct TLRa compositions, leading to tunable activation of each TLR signaling*