# Discovery and evaluation of bicyclic peptides as modulators of pattern recognition receptors

Abstract #

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## ABSTRACT

Toll-like receptor 3 (TLR3) is an intracellular pattern recognition receptor aimed at binding double stranded RNA, which leads to cellular activation and proinflammatory cytokine secretion. Modulation of TLR3 biology can have significant impact in oncology and autoimmunity indications. In immuno-oncology, TLR3 agonists have been deployed as adjuvants to activate immune cells such as type I conventional dendritic cells that can help initiate the adaptive immune response to the tumor. In autoimmunity, TLR3 antagonists have been aimed at reducing immune cell over-activation. Despite a potential broad utility, systemic dsRNA TLR3 agonists and existing antagonists have not demonstrated clinical success due to lack of targeting and toxicity resulting from systemic activation. Therefore, the need for new molecular approaches to influence the signaling of TLR3 is evident. *Bicycle®* peptides are small molecules that penetrate rapidly into tissues and solid tumors and have a short half-life compared to biologics, potentially reducing systemic toxicity due to reduced exposure time. Using the *Bicycle®* phage display platform, we have identified several families of *Bicycle® peptides* that bind the TLR3 extracellular domain. *Bicycle® peptides* were evaluated for their ability to modulate TLR3 signaling using a TLR3-overexpressing reporter cell assay. Binding and agonism was also assessed in monocyte derived macrophages that endogenously express TLR3. This system allowed us to more closely evaluate *Bicycles'* ability to bind and modulate TLR3 signaling through known pathways leading to production of pro-inflammatory cytokines.

## INTRODUCTION

- Toll-like receptor 3 (TLR3) is a pattern recognition receptor that dimerizes upon binding of dsRNA.
- TLR3 is typically found intracellularly, but has also been documented to be found on the surface of monocyte derived macrophages.
- Signaling through the TLR3 receptor typically results in the expression of interferon α and interferon β, proinflammatory cytokines, and activation of the immune cell.

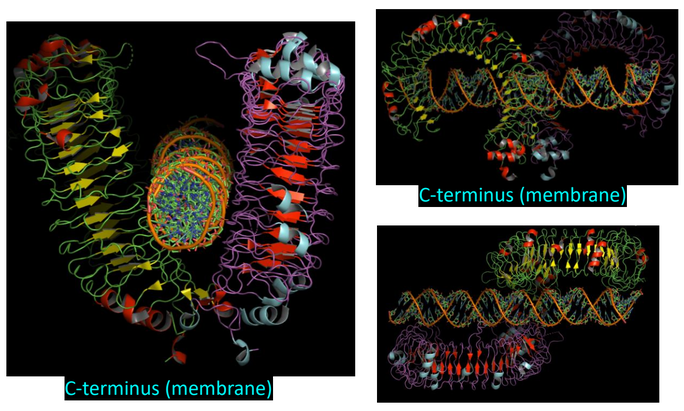


**Figure 1. Crystal Structure of dsRNA bound to dimerized TLR3.** PDB ref: 3CIF

## METHODS

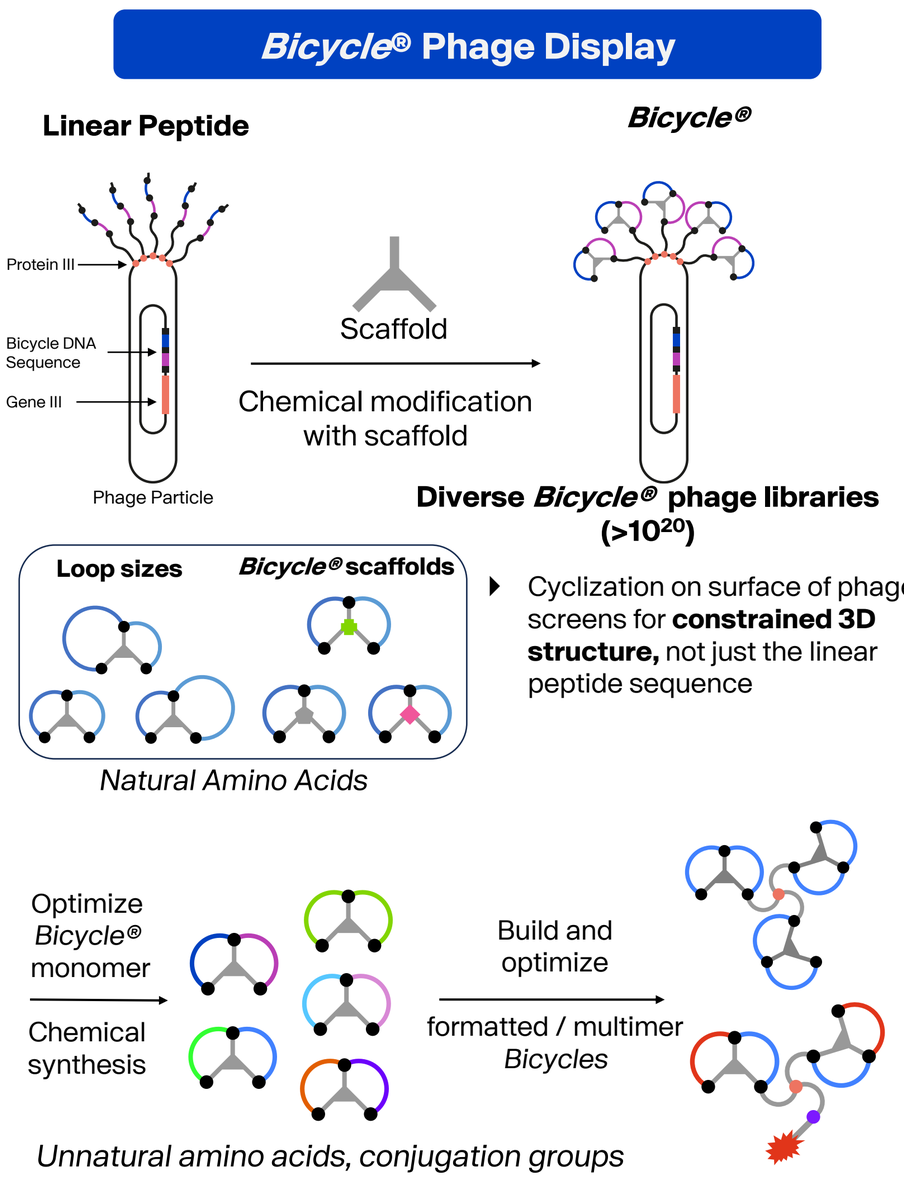


**Figure 2. Phage display screening process and multi-valent *Bicycle®* peptides**

## RESULTS

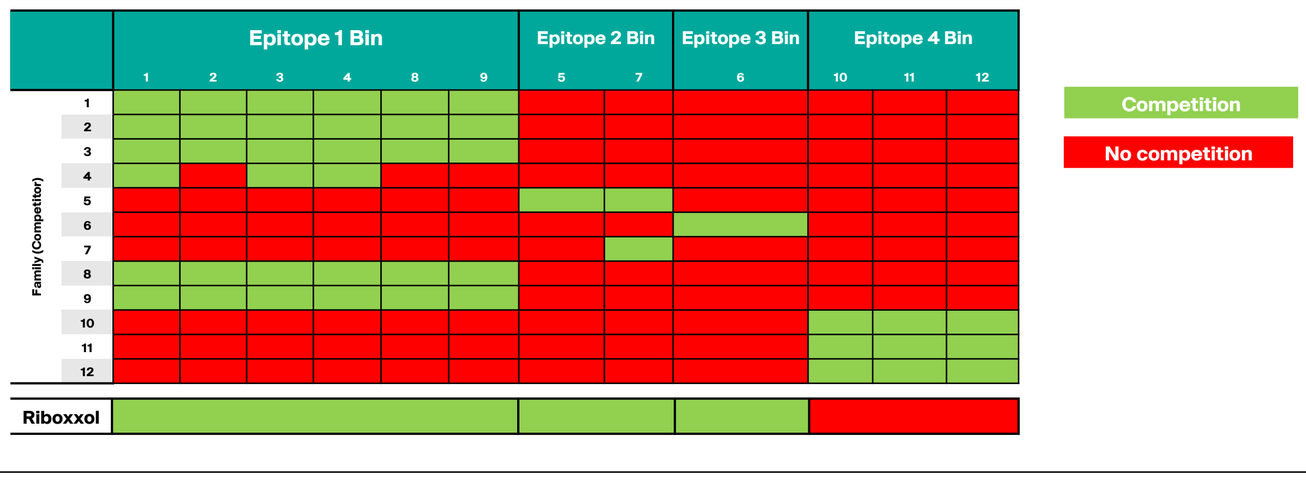


**Figure 3. Phage display generates a diverse set of binders binned across multiple families and epitope bins as defined by alpha screen.** Competition between *Bicycle®* peptides and a synthetic dsRNA, Riboxxol, is observed with Bins 1-3.

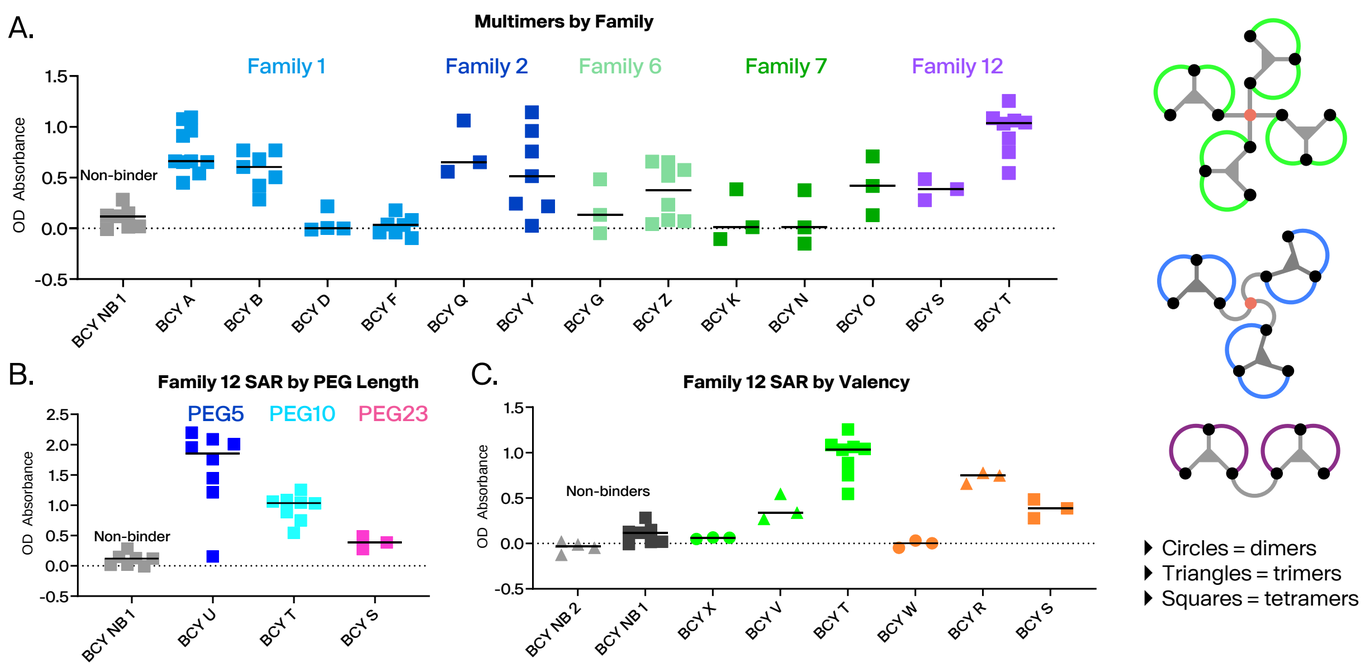


**Figure 4. TLR3 signal transduction is observed across multiple families and *Bicycle®* peptide formats.** A. A survey of families is evaluated for agonism using a reporter cell assay using the HEK-Blue TLR3 Dual Reporter Cell Assay. B. A focused set of Family 12 tetramers with differing PEG lengths was tested, showing the highest activity observed with PEG5. C. When PEG10 (green) is used, valency linearly correlates to activity (tetramer>trimer>dimer); however, when PEG23 (orange) is used as a linker, a different relationship between valency and bioactivity is observed.

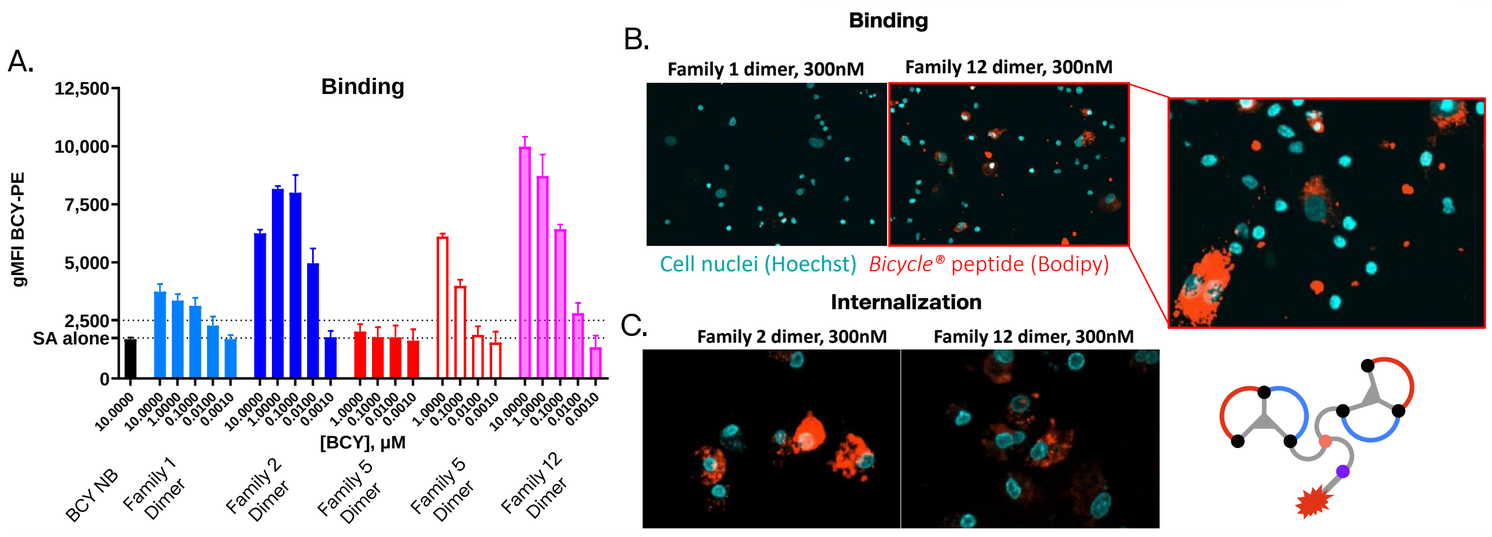


**Figure 5. Binding and internalization to monocyte derived macrophages is observed:** A. *Bicycle* binding is evaluated on monocyte derived macrophages expressing surface TLR3 via flow cytometry. B. Imaging data focusing on Family 12 *Bicycle®* peptides reveals binding (B.) and internalization (C.) to monocyte-derived macrophages.

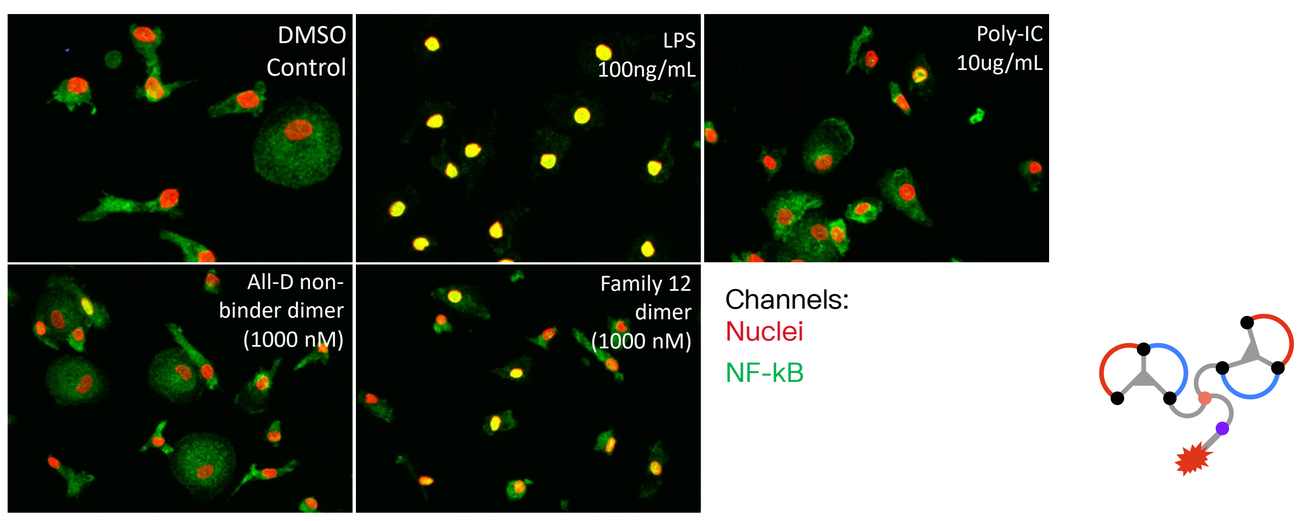


**Figure 6. NF-κB translocation is observed with active *Bicycle®* peptide treatments.** Following treatments with Family 12 *Bicycle®* peptides, NF-κB translocation is observed in monocyte-derived macrophages.

## CONCLUSIONS

- Our phage display platform generated a wide array of *Bicycle®* peptide Binders across 12 families and in multiple epitope bins.
- Functionality differences across families is observed in a signaling assay. Both PEG length and valency influence the activity observed.
- Several families are observed to bind to monocyte-derived macrophages by flow cytometry. Binding and internalization of Family 12 *Bicycle®* peptides is observed in microscopy.
- Family 12 *Bicycle®* peptides show activity by translocation of NF-κB.
- To our knowledge, this is the first demonstration of a fully synthetic peptide TLR3 agonist. This works demonstrates the application of the Bicycle® platform to a very challenging target class.

## REFERENCES

1. Lin Liu et al., Structural Basis of Toll-Like Receptor 3 Signaling with Double-Stranded RNA. *Science* **320**, 379-381(2008). doi:10.1126/science.1155406

2. Leonard, J. N. et. al, The TLR3 signaling complex forms by cooperative receptor dimerization. *PNAS* **105**, 258-263. doi:10.1073/pnas.0710779105

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