Modular Design of Ligand-Toxin Conjugates

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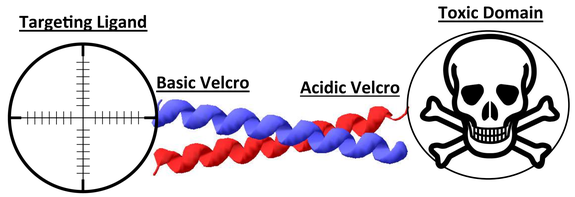
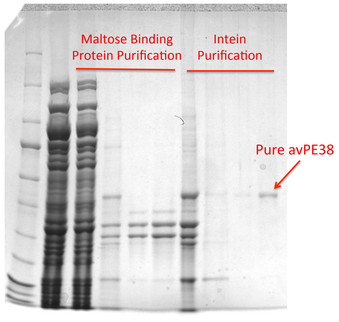
Ligand-toxin conjugates—compounds that link potent cytotoxins to cell-specific targeting ligands—are useful for a variety of applications, from cancer therapeutics to animal chemosterilants. We have implemented a system that separates production of the ligand and toxin domains prior to conjugation, thereby providing versatility in targeting schemes and facilitating rapid testing of ligand-toxin combinations. We anticipate that the system will be used to quickly test hypotheses and screen promising targets for potential drug leads.

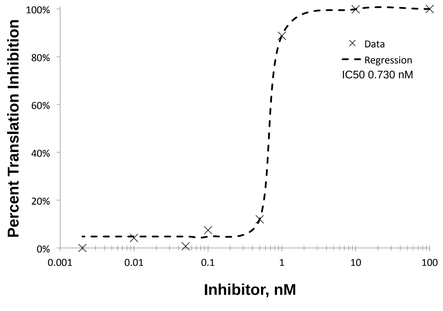
Peptide Velcro for conjugation



A leucine zipper, composed of acidic and basic “Velcro” peptide sequences (O’Shea *et al.*, 1993), is used as a heterodimerization domain to fuse ligands with the *Pseudomonas* exotoxin fragment, PE38. PE38 is produced in *E. coli* as a recombinant protein fused with the acidic Velcro sequence (av-PE38). This protein is immediately amenable to conjugation with any protein, peptide, or small molecule ligand that harbors the partnering basic Velcro (bv) peptide.

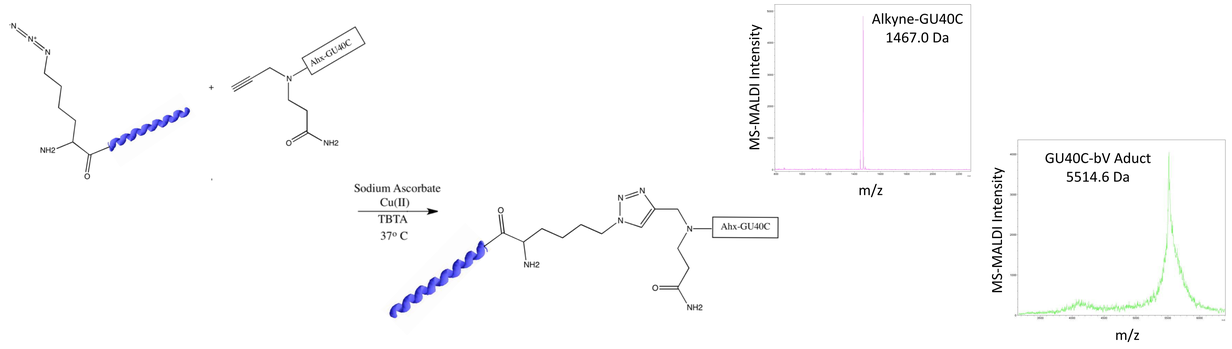
Toxin-Velcro fusion retains full potency





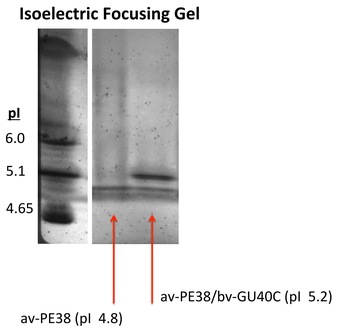
av-PE38 is produced with N- and C-terminal affinity purification tags which are ultimately cleaved off to produce the purified full-length toxin. av-PE38 has an IC_{50} of 0.73 nM, comparing well to literature values of 0.1–10 nM using a cell-free protein translation inhibition assay.

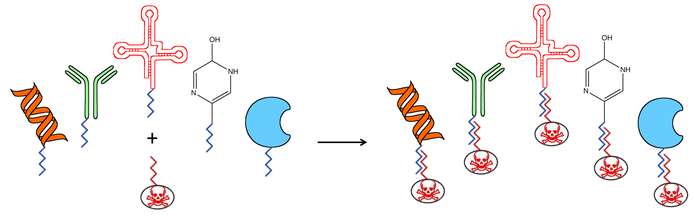
Click chemistry for rapid ligation of ligand and basic Velcro



A synthetic bv-peptide with an alkyne-reactive azide takes advantage of click chemistry for rapidly porting different ligands to our toxin conjugation system. Here, we show ligation of the bv peptide to a peptoid antagonist of VEGFR2, GU40C.

Modular design allows mix-and-match conjugation





Mixing equimolar av-PE38 and bv-GU40C results in >90% yield of ligand-toxin conjugate. The bv-azide allows any alkyne-containing molecule to be quickly conjugated to a toxin of choice, enabling the creation of large and diverse libraries to be generated and tested.

Acknowledgments

This work was supported by a Michelson Grant from the Found Animals Foundation. Requests for reagents can be made by contacting the PI (wja@scripps.edu).