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Title:	Identification of venom-neutralizing nanobodies and developing a rapid nanobody selection platform
Authors:	Dubey, Abhishek
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Abstract:	In this study, we have attempted to identify nanobodies that bind one of the toxic components of Russell Viper Venom (RVV). In addition to this, we have also devised a novel addition to the current bacteriophage display platform that allows rapid identification of nanobodies. In Chapter 2 of this study, we identified a nanobody that specifically binds Fr2(RVV) with high affinity of ~ 8nM. The nanobody also partially neutralizes toxicity induced by RVV in the zebrafish model system. When used in combination with a previously reported anti-RVV nanobody, MKSS1, FR2-60 nanobody was able to further extend the survival of the intoxicated fishes. We also investigated different types of genetic linkers that could be used to make bispecific and bivalent nanobody conjugates. In Chapter 3 of this study, we describe a novel addition to the current phage display technology platform that allows rapid and swift isolation of antibody fragments by coupling phage display with flow cytometry. We utilize the ability of fluorescent DNA binding dyes to specifically label the bacteriophage genome which allows their easy visualization on a flow cytometer under standard conditions. We then utilize the ability of fluorescently labelled bacteriophages that display the entire nanobody library to perform biopanning using a cell sorter. The antigen, H2-K b :β2:SSIEFARL was fluorescently labelled by conjugation with Streptavidin-PE and was used for sorting bacteriophages that display nanobody which binds to H2-K b molecules. Apart from this, we also describe a separate platform to identify agonist and antagonist nanobodies against target membrane protein.
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