Abstract

The resurgence of V H H single domain antibodies is considered as a breakthrough in the field of antibody engineering as it broadens the scope to develop cost-effective, globally applicable therapeutic proteins that can be custom modified for use in the field of diagnosis, research and treatment. A phage library containing phages displaying V H Hs obtained from the whole blood of a naive Indian Bactrian Camel was generated and maintained in the lab. Commercially available PPRV specific monoclonal antibodies are expensive and has limited scope in terms of diagnostic and therapeutic applications. So we performed biopanning for PPRV live attenuated form of virus and 2 V H Hs were selected after phage ELISA for protein level analysis.

The V H H from the phagemids selected on the basis of phage ELISA can be customized by insertion of tags that serve as the retrieval handles if these binders are used as detecting agents.

Therefore, the experiments were planned to modify the expression vector, pET22b(+) containing V H H with a 6X(HIS). A thrombin cleavage site (TCS) and a Biotinylation tag (BT) were inserted downstream to V H H but upstream to 6x(HIS). A vector construct facilitating the downstream biophysical characterisation of the selected V H H s was generated successfully and was confirmed by sequencing. The activity of biotinylation tag was established through western blot of the biotinylated protein using HRP conjugated streptavidin. The failure of the recombinant protein product to get segregated on incubation with Ni-NTA beads indicates a probable activity of bacterial, endogenous serine proteases, which should be tested and confirmed. The vector thus modified was labelled as BNT for expressing protein that can be used for biotin-streptavidin based pulldown assays, Ni-NTA sensor based binding kinetics of V H H with the specific antigen, generation of tetrameric versions etc... Biopanning was also performed to screen V H Hs specific against GPR114, which is suspected to be an immunologically important receptor protein. All the 5 V H Hs were sub cloned into Vector BNT for the ease of downstream characterization of the proteins. As the activity of bacterial serine proteases would cleave off the 6XHis tag of completely refolded V H H, while in cytoplasm; protein purification based on 6XHis tag is feasible only from inclusion bodies. The refolding conditions of thus purified J6 and J20 proteins should be optimized and further characterization can be performed. The proteins were induced in large scale and purified from inclusion bodies.Extensive screening should be done for selecting V H Hs from the phages obtained on biopanning of GPR114.