

DIRECTIONAL SANDWICH PANNING IN SOLUTION TO SELECT INHIBITORY NANO-ANTIBODIES AGAINST FRAGILE ENZYMES INCLUDING PROTEASES

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Enzymes may represent a major target in drug discovery programs. Potent and specific enzymatic inhibition can be achieved by single domain antibody fragments (referred to as Nanobodies®, Nano-Antibodies or VHHs) with higher specificity and optimized pharmacokinetic parameters compared to small molecules. The selection of inhibitory VHHs from large repertoires by phage display is challenging since (i) the active site of the target enzyme needs to be accessible by the phage-displayed VHH and (ii) the lifetime of active enzymes can be too short for efficient enrichment of inhibitory VHHs over successive rounds of biopanning. To tackle these challenges, it is crucial to optimize panning and screening strategies that facilitate the selection of such inhibitory VHHs. Here, we demonstrate that a directional sandwich panning in solution (DSPS) with the target enzyme immobilized via a selected biotinylated target-specific, non-inhibitory VHH on the surface of streptavidin paramagnetic beads allows the isolation of high affinity competitive inhibitory VHHs (K_D comprised between 2 and 36 nM) against two fragile enzymes (i.e. a serine protease and a beta-lactamase). Conversely, the selection of such inhibitory VHHs was not possible by means of conventional pannings against target enzymes passively adsorbed on ELISA plates. The critical step for successful DSPS is the prior selection of a tailored capturing VHH that stabilizes the active enzyme and orientates its active site to the VHHs library displayed on phages. These results demonstrate the great value of DSPS to facilitate the selection of highly potent enzyme inhibitory VHHs.