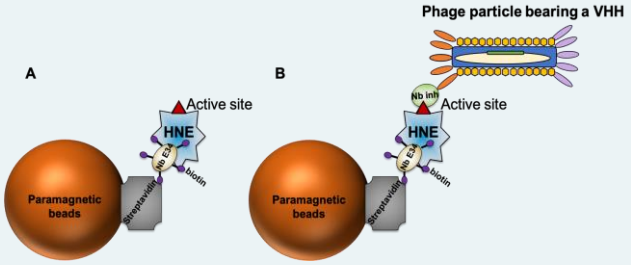
Method for the isolation of single domain antibodies (VHH) binding to pre-selected epitopes

METHOD

Selection of binders to a **precise epitope** by functionalization of streptavidin magnetic beads with a biotinylated single domain antibody (VHH).



A. *Functionalization of streptavidin beads.* ***B****. Capture of phages bearing VHH against the active site of the enzyme.*

KEY ACHIEVEMENTS

- Isolation and characterisation of VHHs inhibitors binding to the active site of human neutrophil elastase (hNE, first VHH described to date) and the New Delhi metallo-beta lactamase-1 (NDM-1)
- Binding of hNE inhibitors to the selected epitope confirmed by crystallography.

KEY COMPETITIVE ADVANTAGES

- Phage/ribosome and yeast display often results in unspecific binders or binding to irrelevant epitopes for downstream applications. Epitope-specific directional immobilization on magnetic beads overcomes these limitations by exposing selected epitope(s) to the repertoire.

- Epitope-specific orientation of the target protein results in exposure of specific epitopes (e.g. the active site of enzymes) thar are not presented by passive adsorption or biotin-streptavidin immobilization.

- VHHs are more specific than antibodies and streptavidin functionalized beads and no batch-to-batch variations are encountered, as is regularly the case with conventional antibodies.

While the selection of single chain antibodies (VHHs, also known as Nanobodies®) binding a target protein by phage or yeast display is relatively straightforward, the generation of binders with a specific biological activity or against a pre-selected epitope can be cumbersome.

Immobilization by passive adsorption on the surface of ELISA plates leads to random orientation through multiple unspecific non-covalent interactions and partial denaturation of the target protein. Immobilization via biotin-streptavidin is more efficient but relies on the biotinylation of the protein, which might impair its activity and structure.

To tackle these problems during the selection of specific binders from libraries, we have developed a **directional sandwich panning in solution (DSPS)**. To validate this method, we spiked an immune library with phagemids coding for an artificial hNE competitive inhibitory single domain antibody (VHH-H7SP1-N). Then, we performed three rounds of panning selection either with the DSPS using the non-inhibitory VHH VHHE34 as hNE capture agent or by passive adsorption of the hNE on ELISA plates. The VHH-H7SP-1N was isolated only with the DSPS strategy and not in the panning performed on ELISA plates. The same approach was successfully used for the selection of VHHs inhibiting NDM-1. These results indicate that the DSPS for epitope-specific orientation of the target protein selects binders that are not found when the selection is performed by passive adsorption immobilization.

UPCOMING CHALLENGES

- Validation of selected hNE inhibitors in relevant preclinal models.
- Validation of the method with conventional antibodies.

PARTNERSHIP SOUGHT

- Joint product development
- Technology transfer agreements

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