



Exploring the VHH opportunity

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Orthros Medical

Orthros Medical is developing variable domain of the heavy chain only antibodies (VHH) based therapeutics to meet animal and human patient needs like osteoarthritis (OA), utilizing the unique characteristics of VHH antibody fragments.

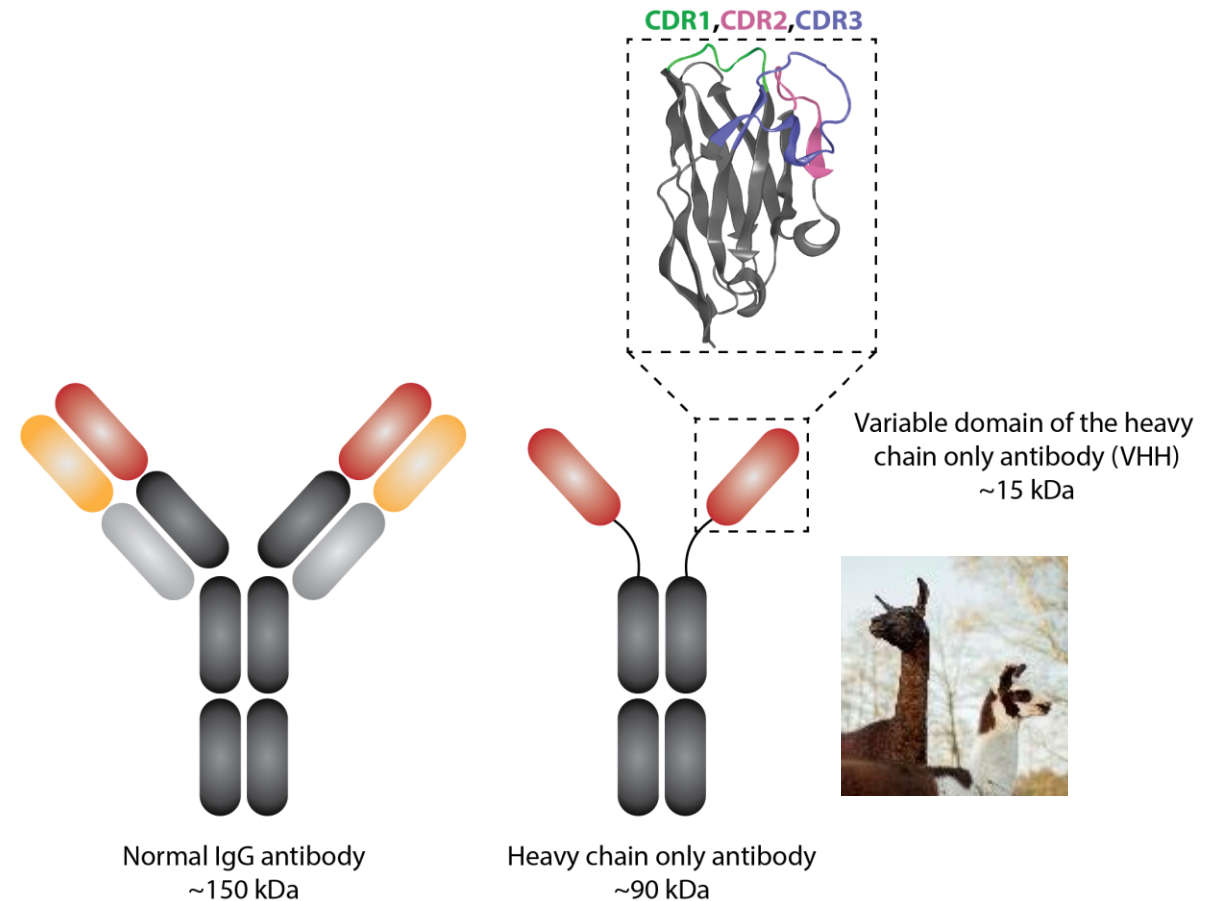
- Situated in “The Green East” in Raalte
- Currently a team of 9 people
 - CEO, Robert Jan Lamers
 - CSO, Prof. Marcel Karperien
 - 3 Senior Scientists with a PhD
 - 4 Lab technicians
- Fully equipped GDP development and manufacturing lab





Introduction of VHH

- Conventional antibodies (IgG)
 - Heavy chain and light chain
 - Constant and variable domain
- Heavy chain only antibodies (HCAbs)
 - Heavy chain only
 - Constant and variable domain
- Variable domain of HCAbs; VHH
 - Full antigen binding domain
 - Engineered and recombinantly expressed
 - Bacteria; *Escherichia coli* (*E. coli*)
 - Yeast; *Saccharomyces cerevisiae* and *Pichia pastoris* (*S. cerevisiae*, *P. pastoris*)





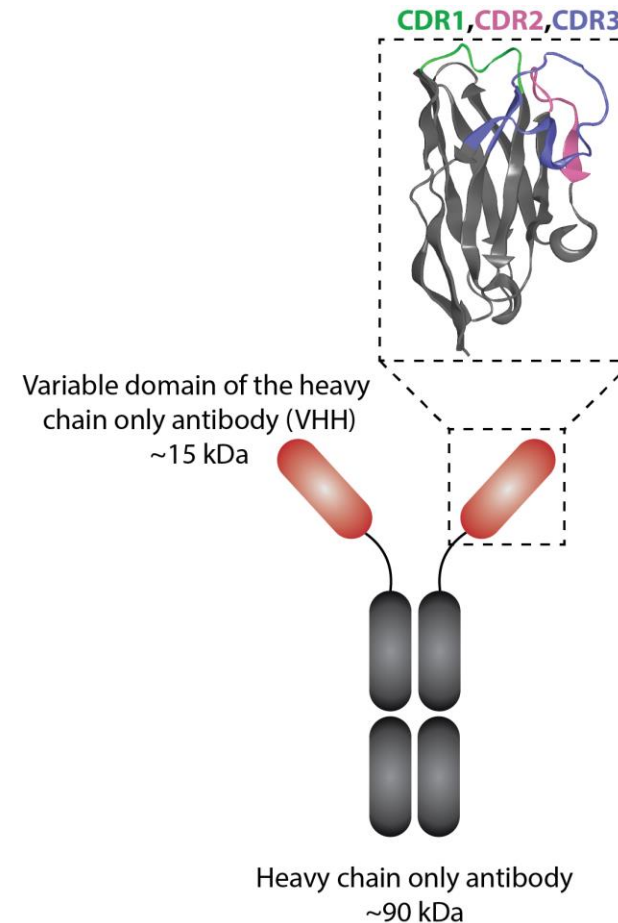
Introduction of VHH

- Advantages
 - Small size; deep tissue penetration
 - Thermostable, soluble
 - High biological availability
 - High binding affinity (*low pM to nM range*)
 - Easy to access more difficult epitopes (*CDR3*)
 - Low immunogenicity risk profile
 - Easy to manufacture in microbial cells
 - Low production costs
- Disadvantage
 - Rapidly cleared, but ...
 - Great safety profile
 - Interesting for diagnostics/imaging (*fluorophores, radioactive isotopes*)

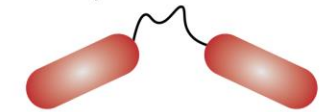


Engineering possibilities VHH

- Multivalent VHH constructs
- G-S linker from C-term to N-term
 - Variable lengths available
 - Genetic fusion; no complex chemistry
- Tags
 - Cys-FLEA for maleimide thiol chemistry
 - Site specific for fluorophores, biotin, biomaterials, ...
 - Tags for improved purification
- Formatting may improve
 - Binding affinity
 - Specificity
 - Valency
 - Tailored half-life



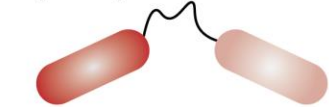
Monospecific bivalent VHH



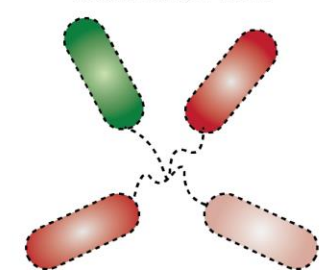
Heterospecific bivalent VHH



Biparatopic bivalent VHH

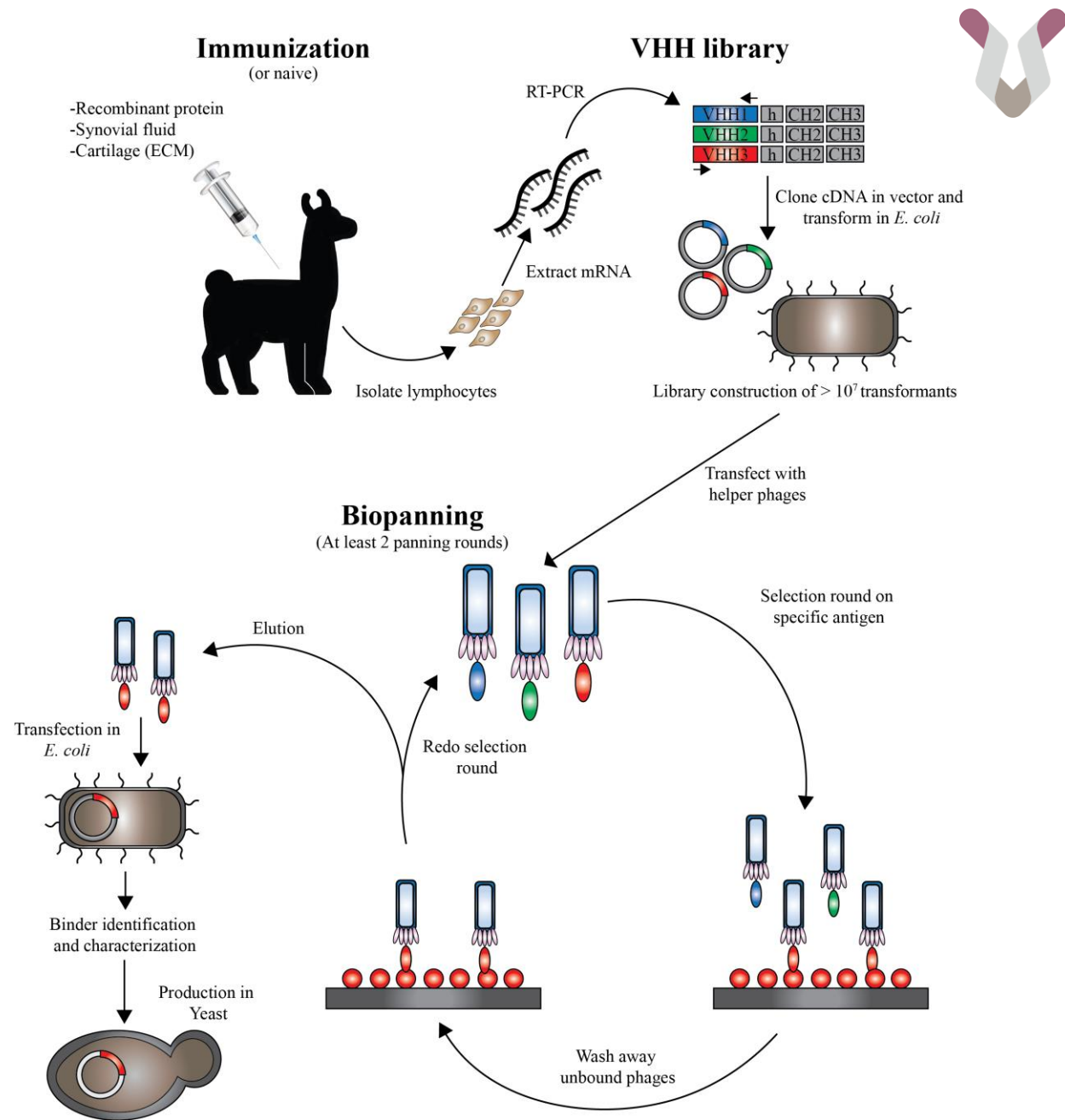


Multivalent VHH



Production of VHH

- Orthros outsources part of the procedure to derive potential VHH clones
 - From immunization to biopanning
 - Initial production in *E.coli*
- Output biopanning rounds is sequenced by next generation sequencing (NGS)
 - In depth NGS analysis
 - Dedicated cloud-based bioinformatics software
 - Short list with potential VHH clones
- Further characterization at University of Twente
 - Affinity
 - Neutralization
 - Cell binding assays
 - Reporter cell assays

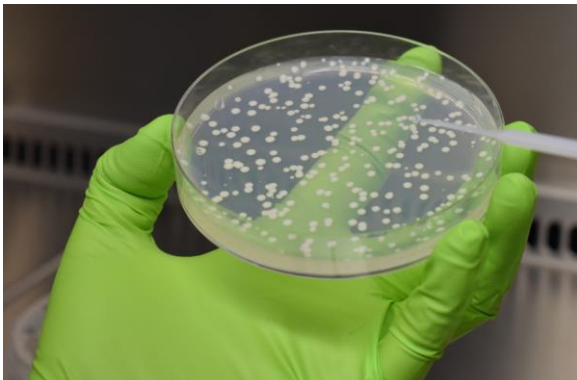
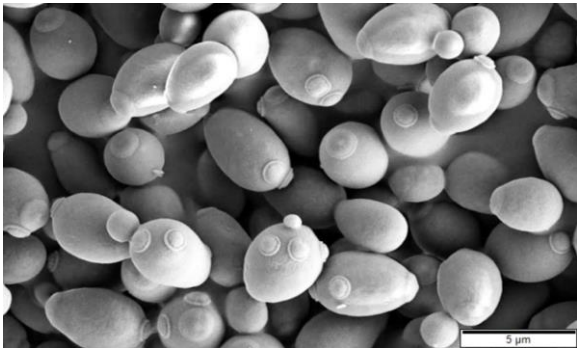




Productions at Orthros Medical

Yeast

eukaryotic organism used as expression system for VHH production

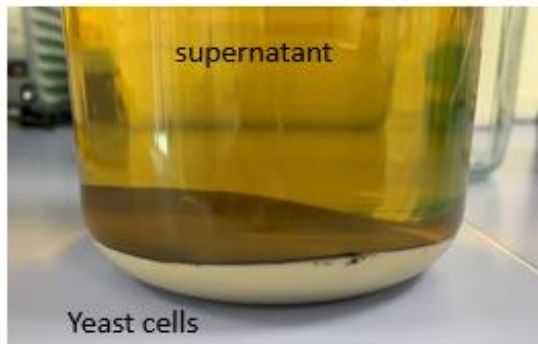


- *Saccharomyces cerevisiae*
/early screening of clones and their formats/
- *Pichia pastoris* (current name *Komagataella pastoris*)
/leading clone production/
- GRAS status (Generally Recognized As Safe)
- Integrate plasmids to their genomes
- Both with a long history of industrial application



Productions at Orthros Medical

- Clones selected for scaled-up productions:
 - 2L shake flask
 - 5L fermenter
 - Further scalable to 50L, 250L and 1000L fermenters





Opportunity in LoAD

- Focus on (disease modifying) osteoarthritis drugs
 - Potential new targets
 - Impact of inflammation on mechanotransduction
 - Important markers
- Replacing, reducing and refining (3Rs) principles in medicine testing
 - Better screening of our target before animal testing
 - Screening of potential synergy of combination therapies (e.g., inflammation, pain, regeneration)



Acknowledgements

UNIVERSITY
OF TWENTE.



high | tech | fund BV

