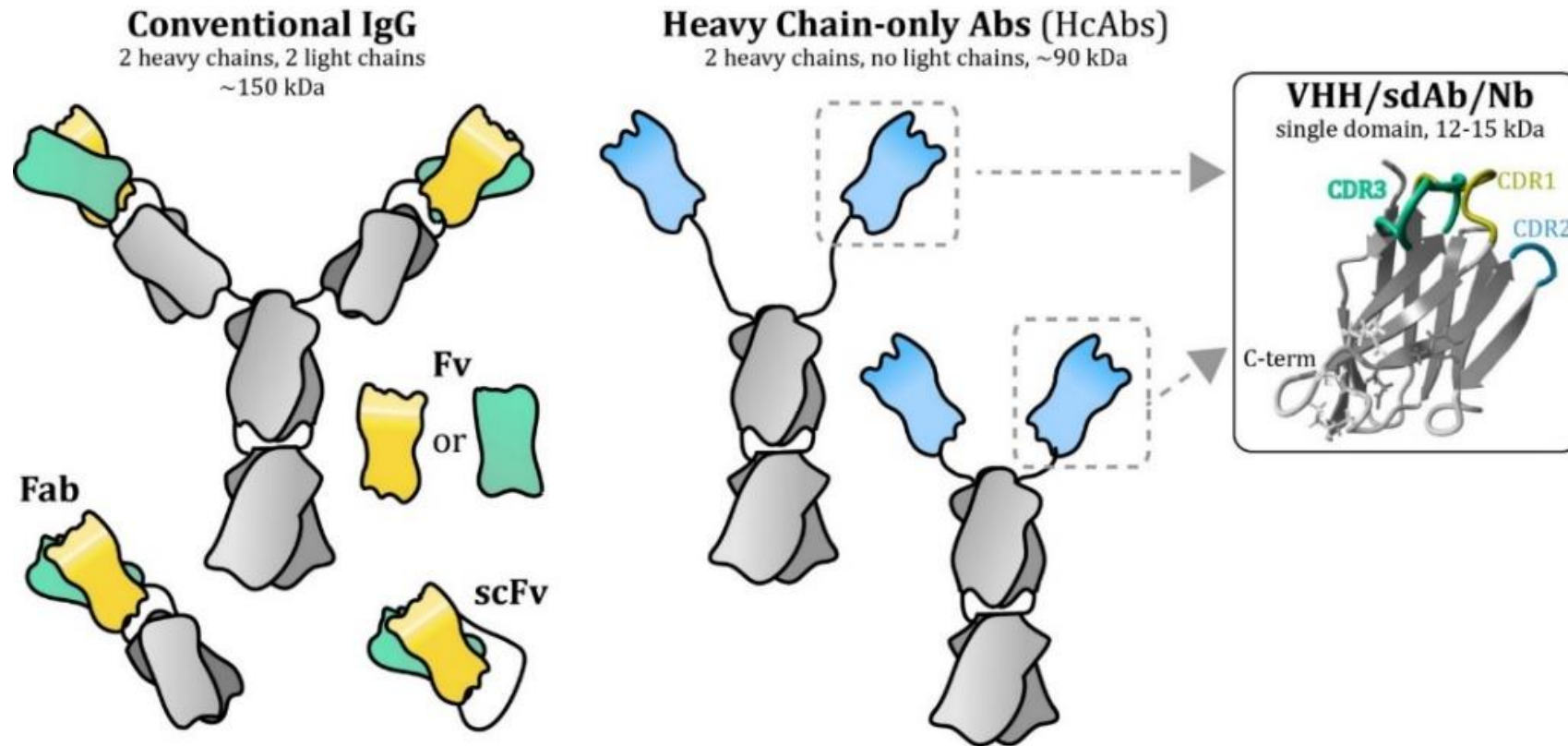


Site-directional functionalization of VHH using the C-Direct tag



**Raimond Heukers, Marjolein Kuijpers, Daphne van Hoesel,
Marta Kijanka, Mohamed El Khattabi, Theo Verrips and Edward Dolk**

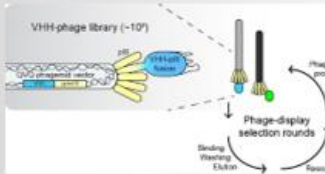
VHH: single domain antibody fragments from camelid-derived heavy chain only antibodies



Services offered by QVQ Holding BV



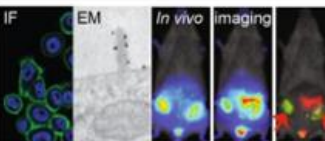
Immunizations
VHH-phage libraries



Phage-display
Panning and selections
Screening



Dose responses
Apparent binding affinities
Potencies



Proprietary C-Direct tag
Directional coupling of:
Biotin, Chelators, Dyes



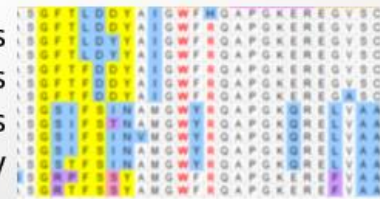
VHH productions in *E.coli*
Purification by means of
IMAC, AC, SEC



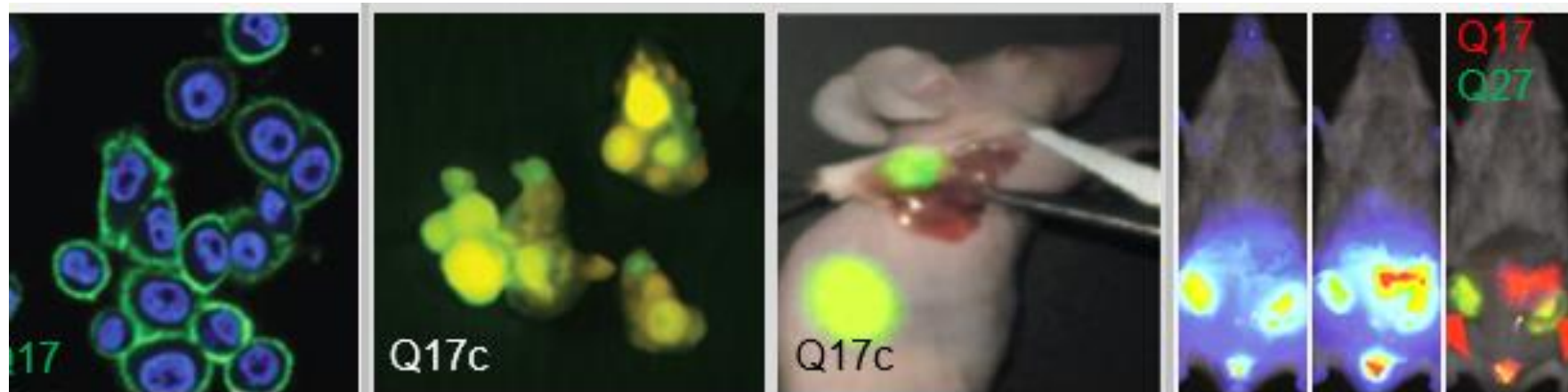
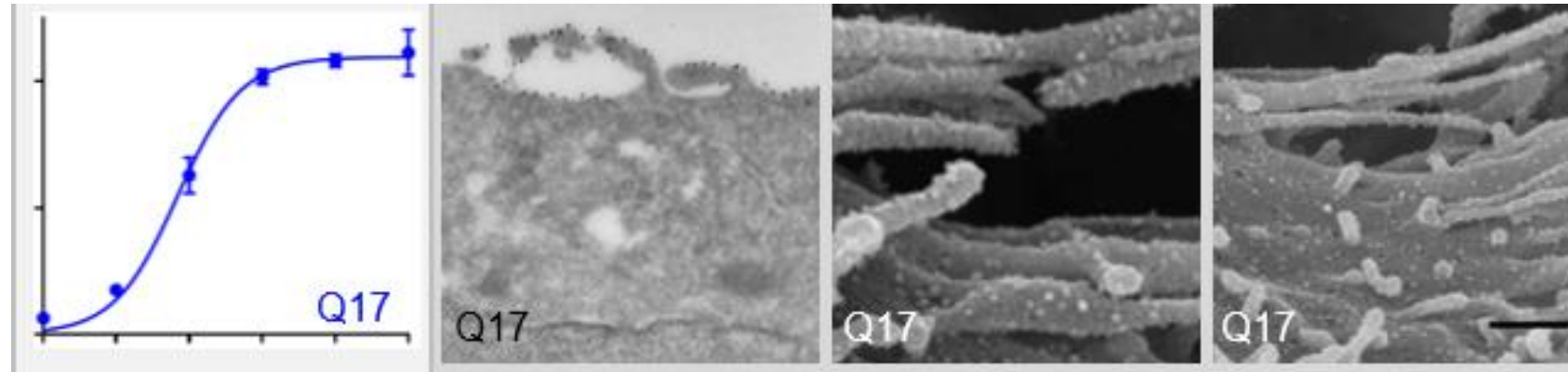
Large-scale productions
GRAS organism *s.cerevisiae*
Shake flask/fermentor



Sequence analysis
Bio-Informatics
Impr. Productions yields
Impr. Stability



VHHs can be functionalized for a large variety of imaging applications



Aim:

**A stable, flexible and
low-immunogenic, C-terminal tag
for directional labeling of VHH**

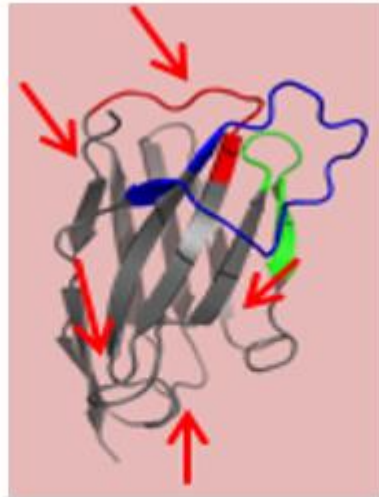


Directional functionalization of VHH retains its binding characteristics



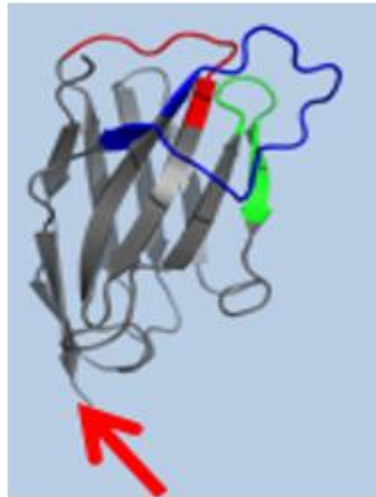
Anti-HER2 VHH
11A4-FLAG-His

Apparent affinity:
~0.4 nM



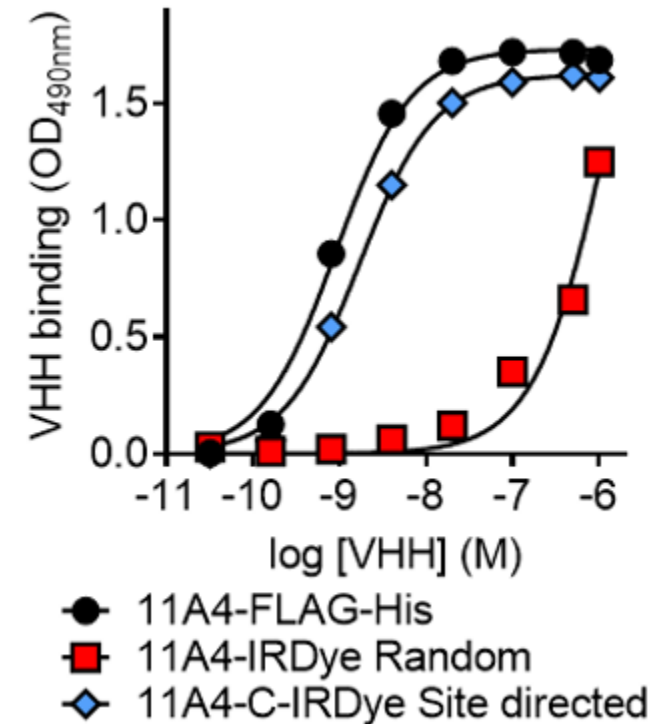
11A4-FLAG-His
Random conjugation to
lysines

Apparent affinity:
>400 nM

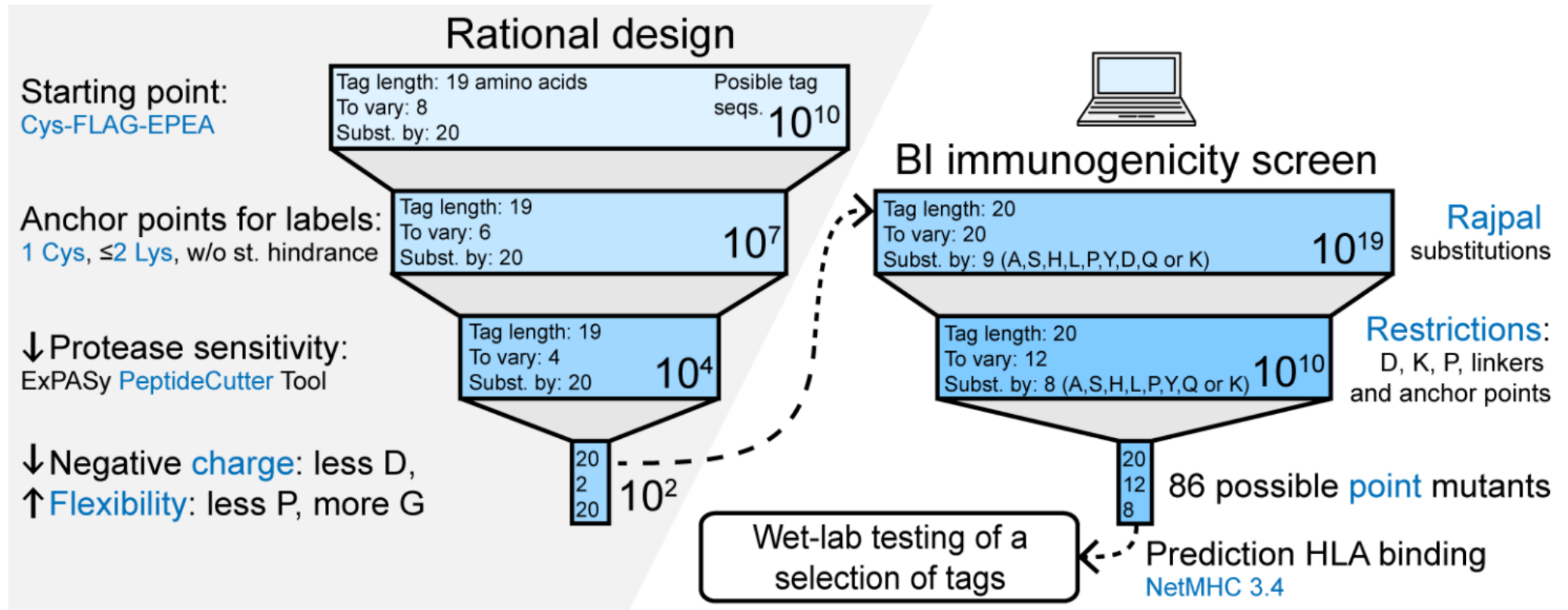


11A4-Cys-FLAG
Site-directional
conjugation to cysteine

Apparent affinity:
~2 nM



Improvement of the cys-FLAG tag via iterations, modeling and Bio-Informatics screens



Differentially tagged VHHs in *E. coli* or yeast production vectors

VHH-FLAG-His

Tag sequence: AAA-DYKDDDDK-GAA-HHHHHH

Vector: pMEK222 Host: *E. coli*



↓ Molecular cloning

VHH-C-Direct

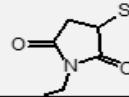
Tag sequence: A-C-A-XXXXXX-EPEA

Vector: pYQVQ11 Host: *S. cerevisiae*



↓ Sulfhydryl-maleimide reaction

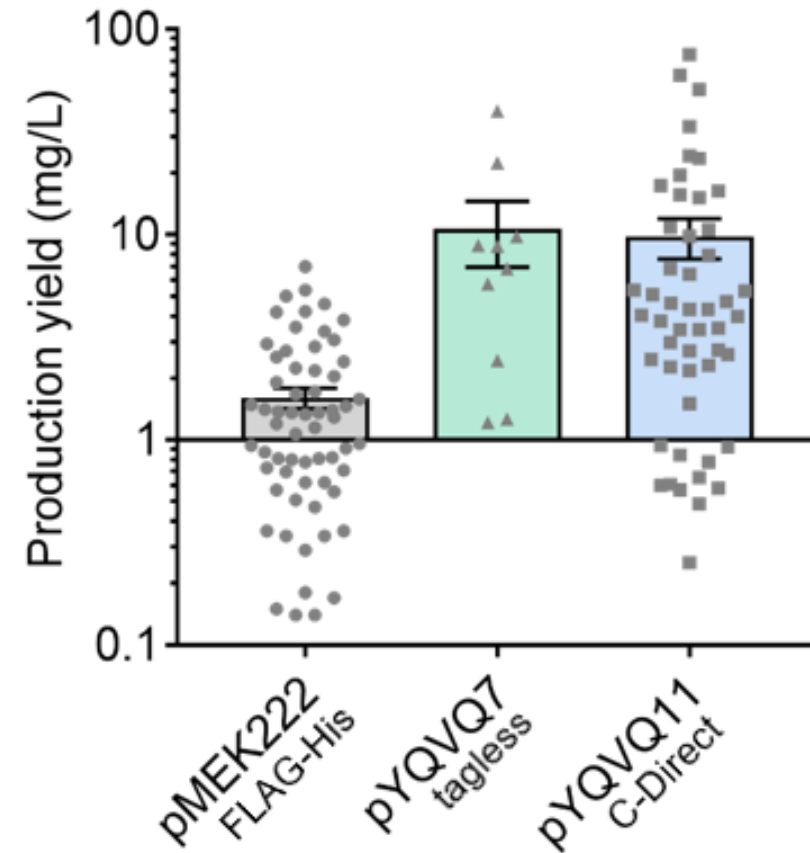
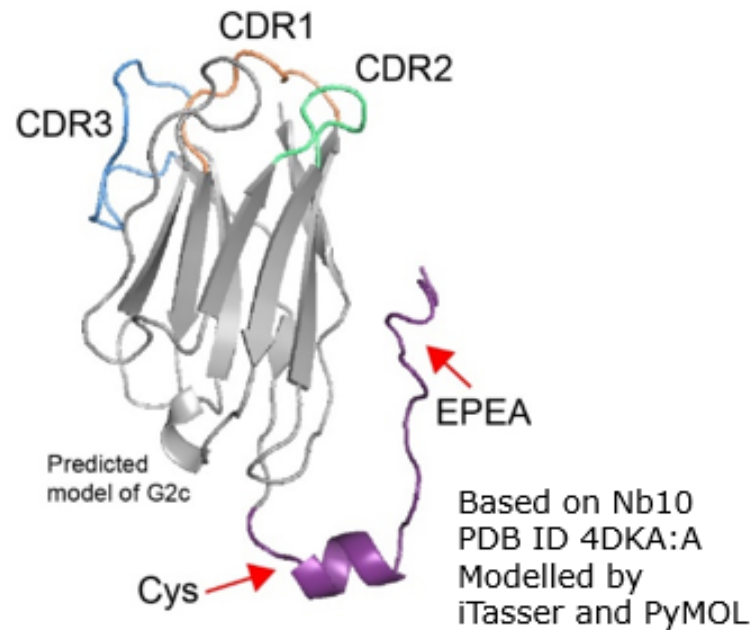
VHH-C-Direct-Labeled



- Biotin
- Chelators (NOTA, DOTA)
- Fluorescent dyes (HiLyte, IRDye)
- Surface or Matrix

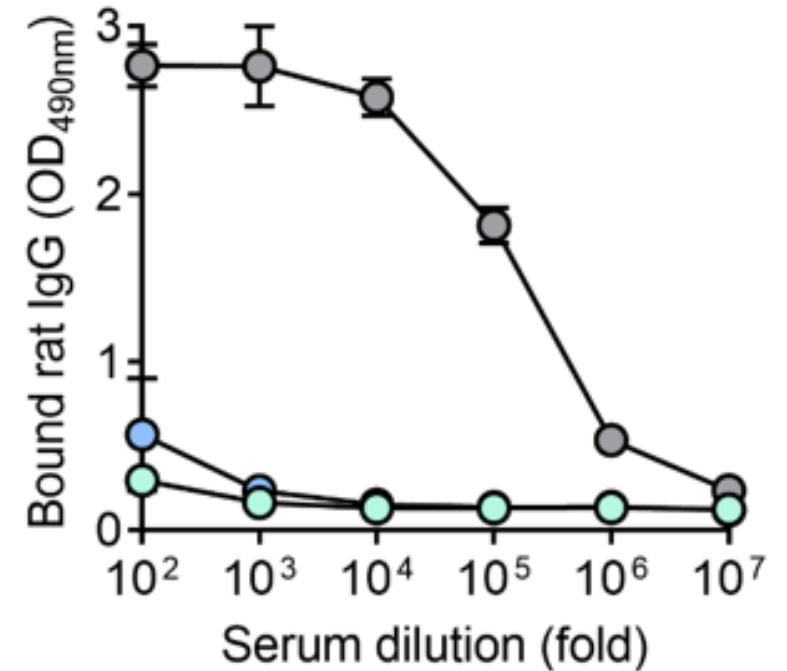
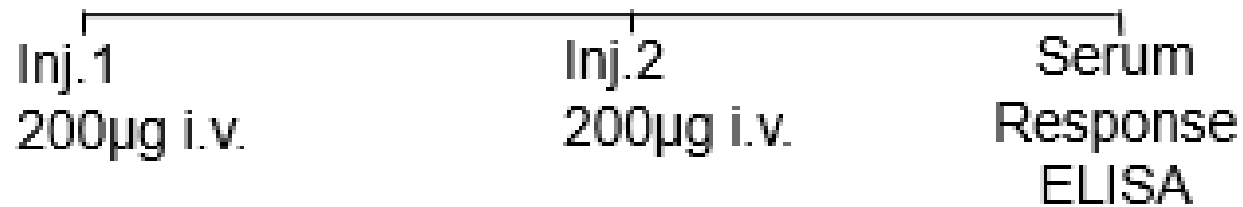


C-terminal C-Direct tag combines free thiol with affinity tag and is produced well in yeast



The C-Direct tag does not induce a significant immune response in rats

Immunisation schedule



- Average resp. after immunization
- Q44-tagless
- Q44-C-Direct



Conclusions

- **VHHs with a C-Direct tag could readily be produced in yeast and purified from yeast supernatant.**
- **Functionalization of VHH via the free thiol in the tag does not significantly affect its binding affinity**
- **Direct conjugation of VHH enabled its detection with a variety of imaging methods.**

