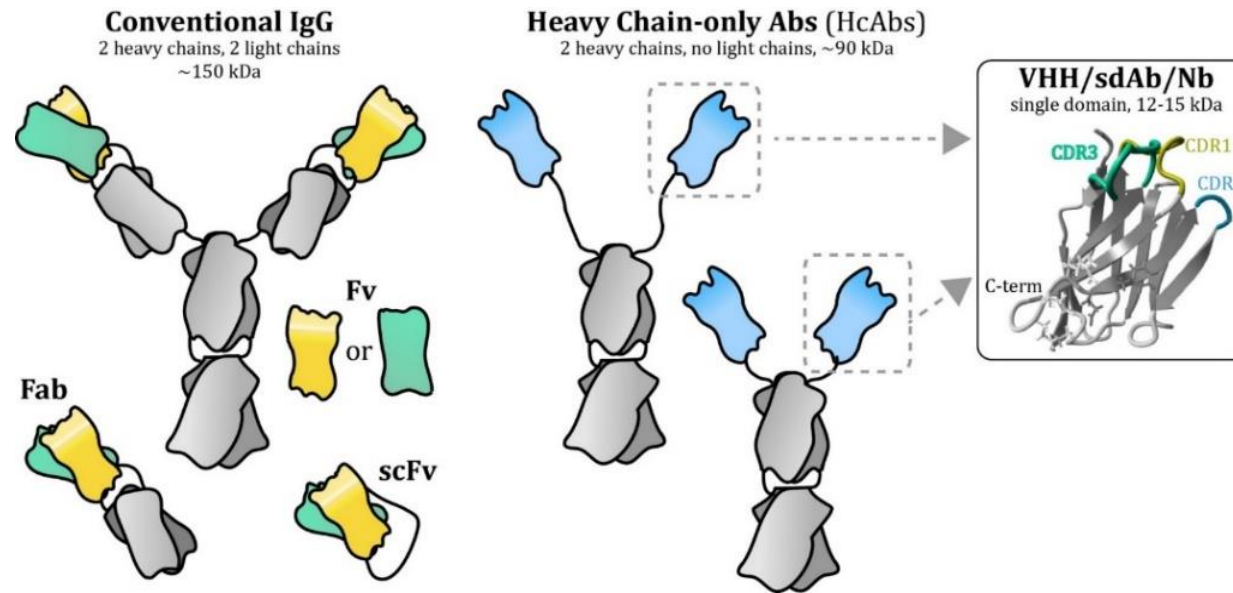


Site-directional functionalization of VHH using the C-Direct tag

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



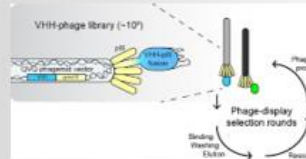
VHH: single domain antibody fragments from camelid-derived heavy chain only antibodies



Services offered by QVQ Holding BV



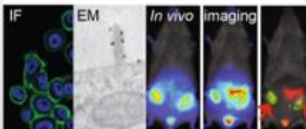
Immunizations
VHH-phage libraries



Phage-display
Panning and selections
Screening



Dose responses
Apparent binding affinities
Potencies



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



Services

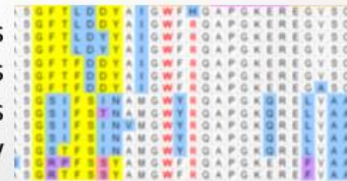
VHH productions in *E.coli*
Purification by means of
IMAC, AC, SEC



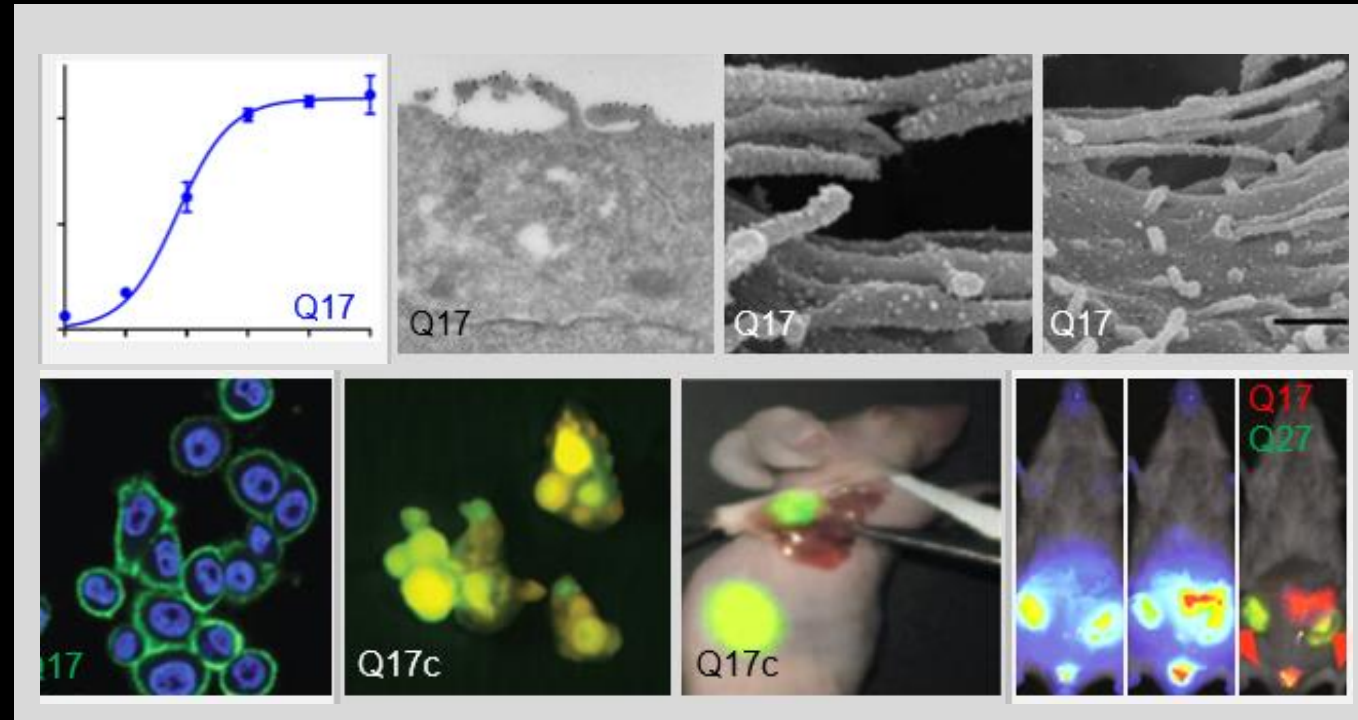
Large-scale productions
GRAS organism *s.cerevisiae*
Shake flask/fermentor



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



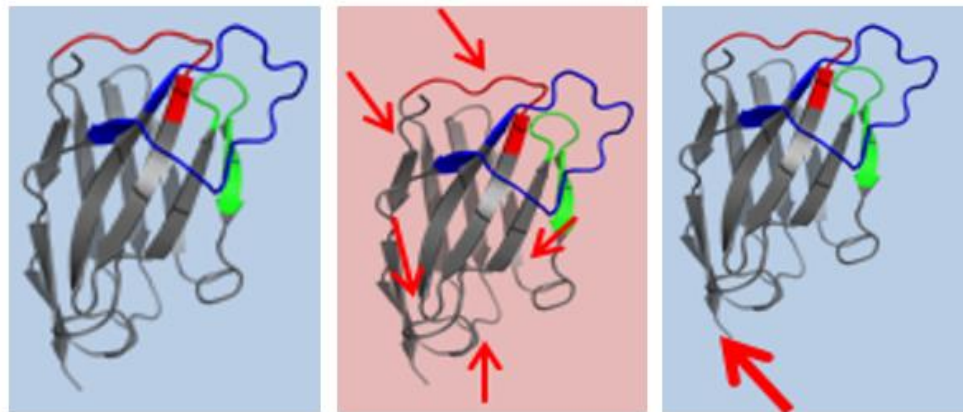
VHs can be functionalized for a large variety of imaging applications



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

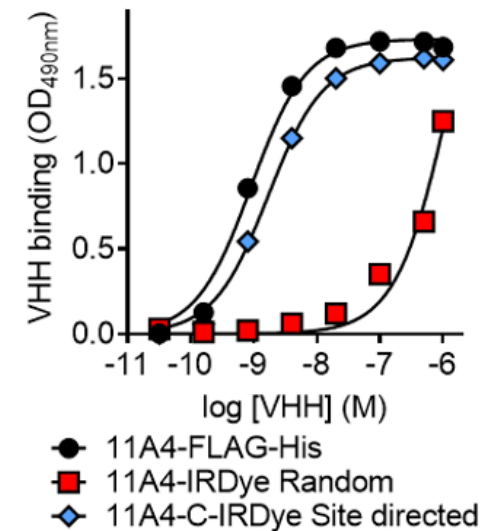
Apparent affinity:
~0.4 nM

11A4-FLAG-His
Random conjugation to lysines

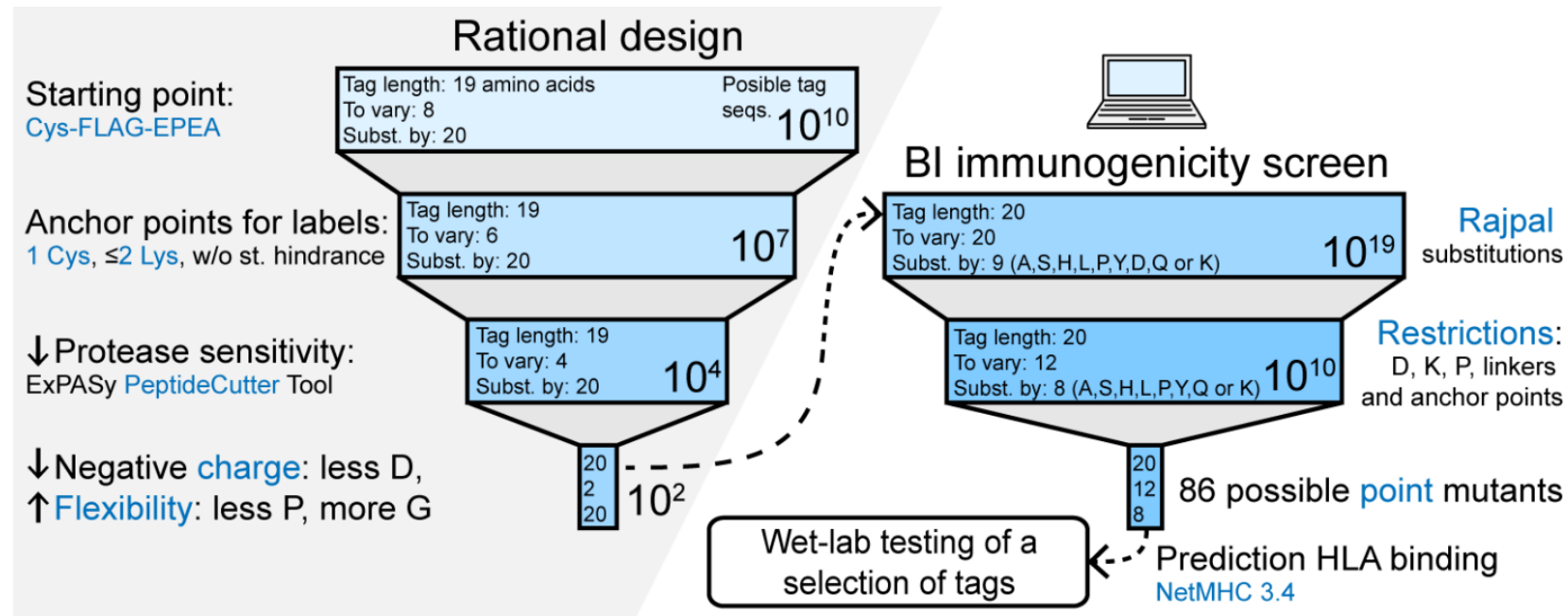
Apparent affinity:
>400 nM

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

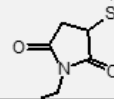
Tag sequence: A-C-A-XXXXXX-EPEA

Vector: pYQVQ11 Host: *S. cerevisiae*



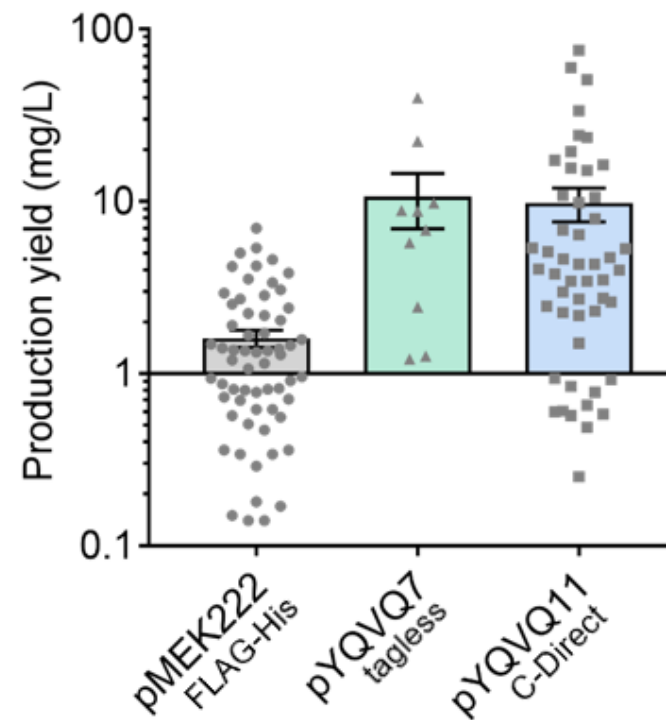
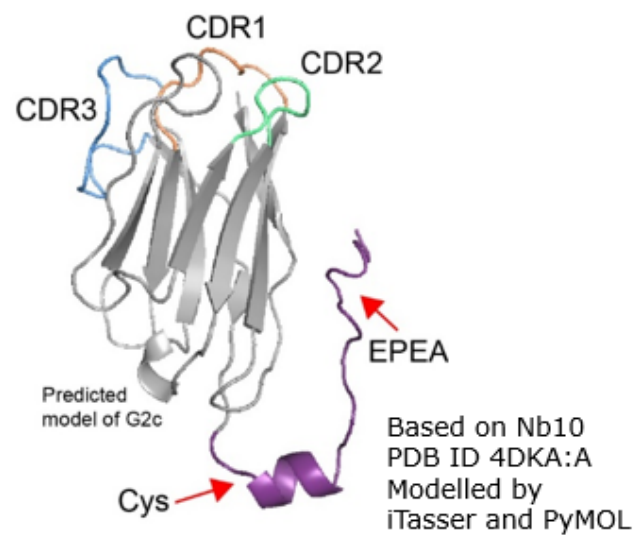
↓ Sulfhydryl-maleimide reaction

VHH-C-Direct-Labeled

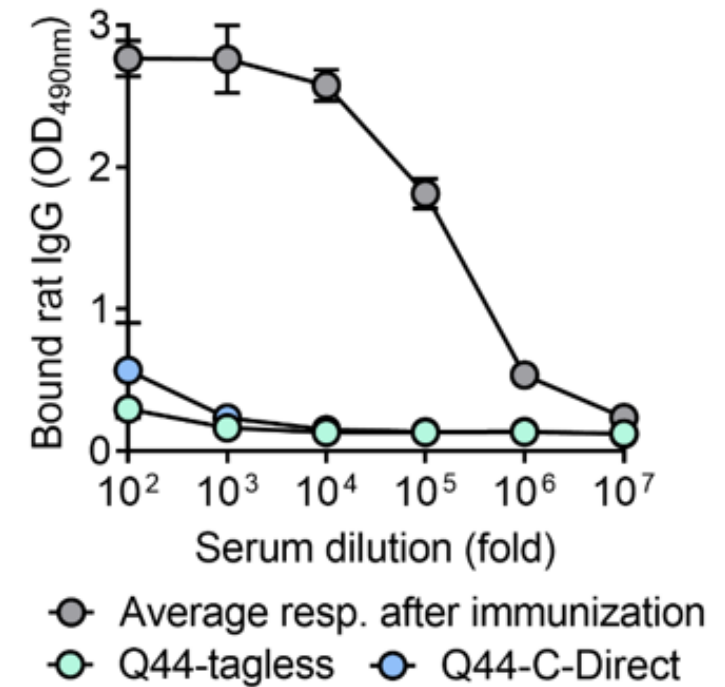
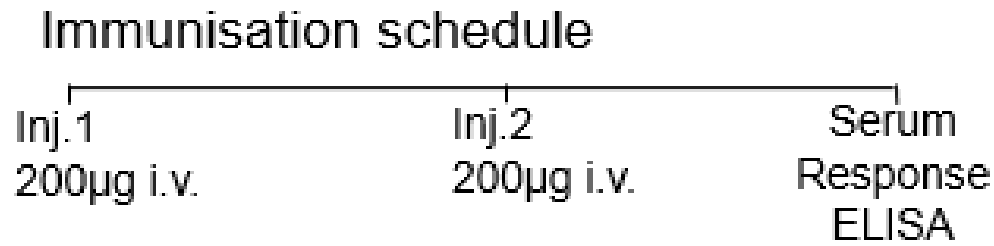


- Biotin
- Chelators (NOTA, DOTA)
- Fluorescent dyes (HiLyte, IRDye)
- Surface or Matrix

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



Conclusions

- VHHs with a C-Direct tag can be readily produced in yeast
- The C-Direct tag can be used to purify VHH from yeast supernatant.
- Functionalization of VHH via the free thiol in the C-Direct tag does not significantly affect its binding affinity
- Direct conjugation of VHH enables its detection with a variety of imaging methods.