Design, assembly and characterization of a humanized scFv framework library

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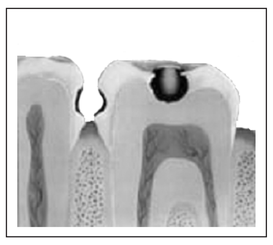
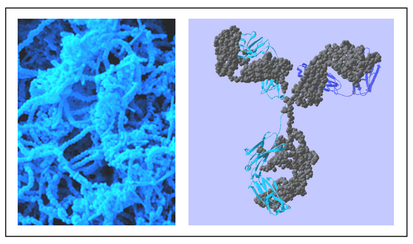
Introduction

The murine mAb Guy 13 binds to the surface protein SAI/II of *S.mutans*, the major cause of dental caries. Used in topical immunotherapy this mAb prevents recolonisation of the tooth surfaces by *S.mutans* and mediates a lasting protection from dental caries.

Humanisation of Guy 13 should reduce the immunogenicity of the antibody in clinical applications to avoid negative side effects and eventually increase stability.





CDR Grafting

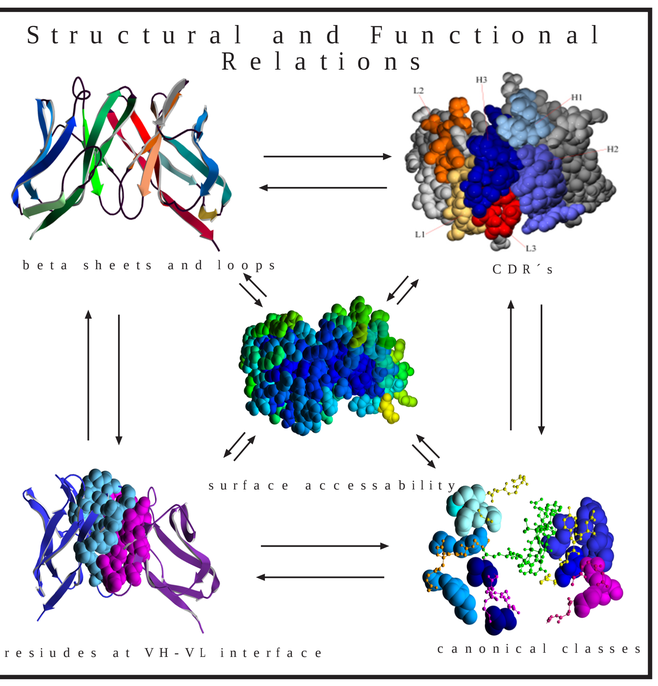
CDR grafting was chosen to humanize the variable domains of the Guy´s 13.

This method transfers CDR´s of a given murine antibody onto a suitable human framework backbone

Selection of the human template sequence is a cruicial step to retain binding activity and characteristics of the antibody

Carefull analysis of the relevant sequences is essential

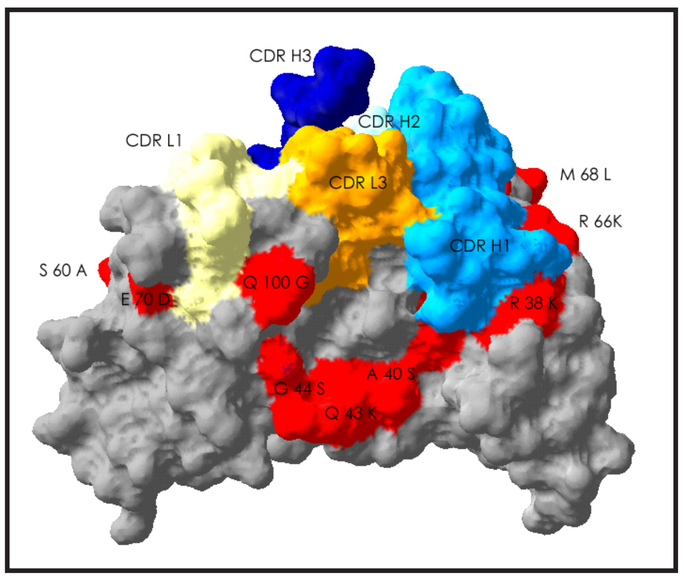
Structural and Functional Relations



Human VH/VL germline gene sequences with a high homology to Guy´s 13 were selected.

24 AA residues with key functions for the structural integrity of the backbone and the CDR loop confirmation were assigned

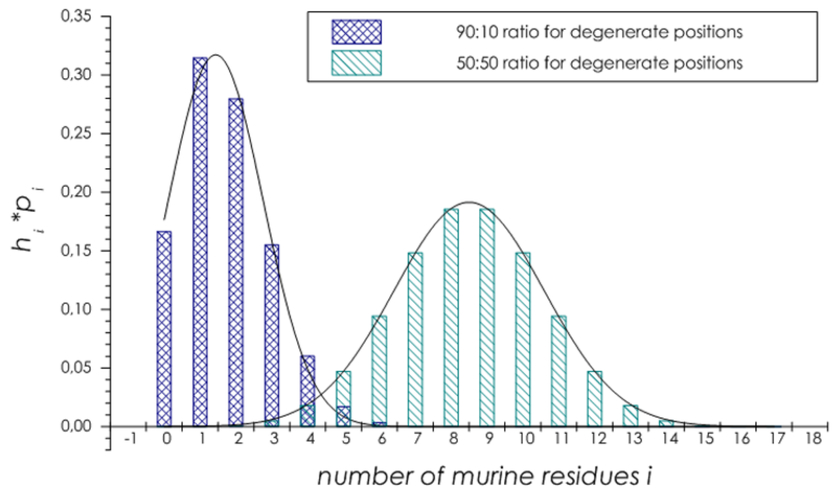
To maximize the chances of creating a fully functional humanized version of the antibody we decided to synthesize a framework library containing mouse and human residues at the critical positions.



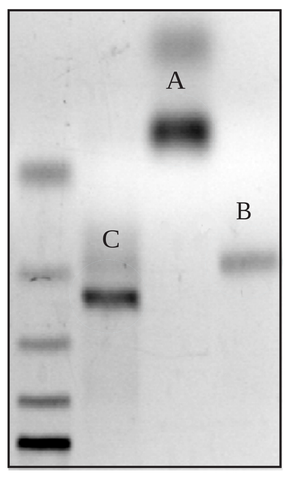
Focused Library Concept

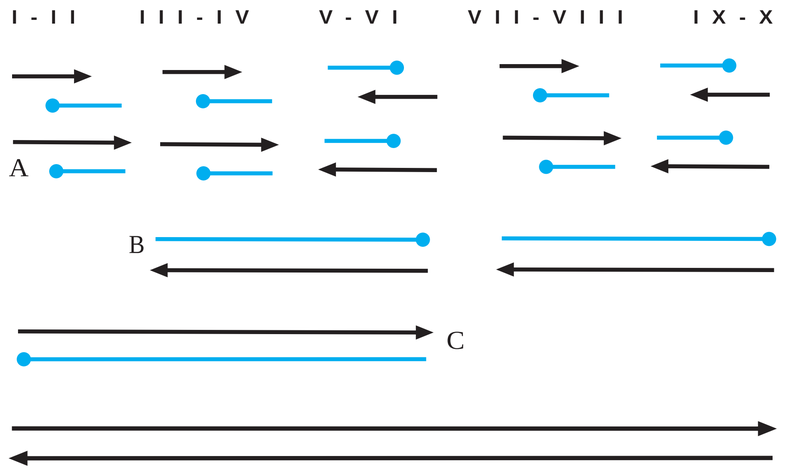
To create a framework library with mouse donor and human acceptor AA´s at the critical positions, degeneracies (wobbles) had to be introduced into the Sequence. Using 50%:50%ratio of degenerate nucleotides at the wobble positions would result in a 1:200 000 chance to find the fully human molecule

By using a 90%:10% ratio we shifted the probability towards the human side of the molecule population in the library and by this a fully humanized scFv can be found in one of 10 molecules



PCR Assembly of the VL Framework library





A VL Framework library was assembled in 4 subsequent PCR amplification steps from 10 overlapping degenerate oligonucleotiodes.

Formation of sideproducts was reduced by mismatches between Oligos and Order of the assembly reactions

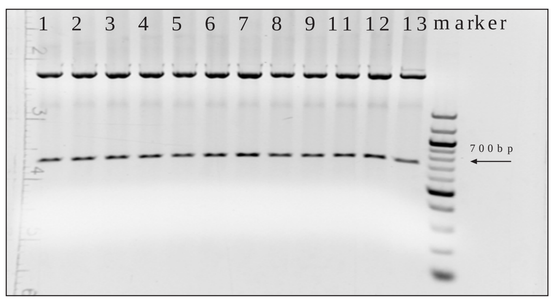
To ensure the complexity of the library and avoid enrichement of certain subspecies through reamplification, equimolar concentrationsof the oligo-nucleotides were used.

Excess oligos and unspecific intermediate products were removed by column purification before use in the next reaction.

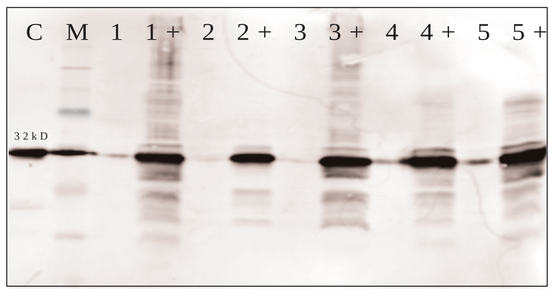
Cloning and Expression

After full assembly of >5ng nanograms of the humanized VL framework library the molecules were joined with the murine VH by convetional SOE PCR and the hybrid library was cloned into a bacteria expression vector and transformed into *E.coli* XL1 blue.

$2x10^6$ clones were obtained, enough to cover the 512 different variations of the VL framework library.



5 randomly picked clones were used for small scale expression. After induction and expression the cell pellets were boiled and run on SDS-PAGE. The scFv´s were visualized in Western Blot by detection of the cmyc Tag.



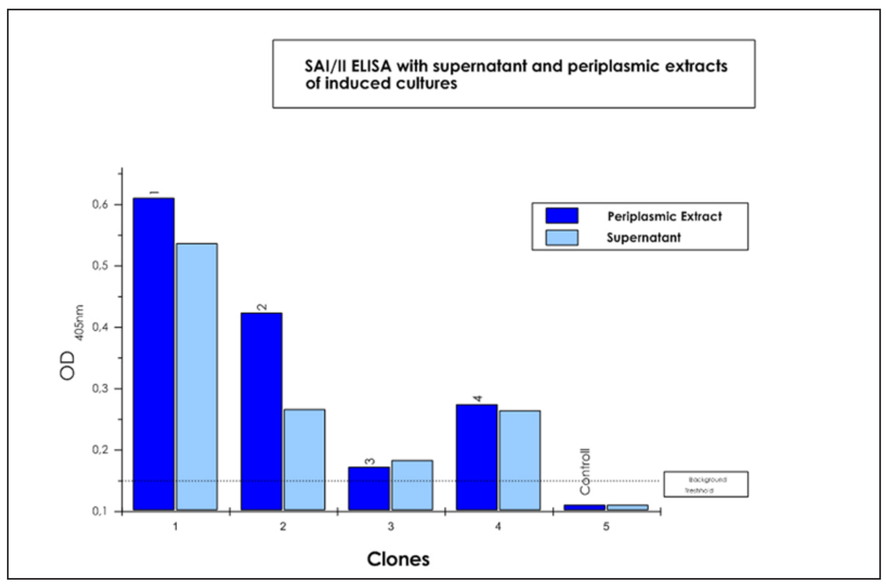
Diversity of the library

Representation of the different degenerate positions in the library were determined by sequencing.

Residue Change	Base Change	Set Ratio	Observed Ratio	Total Number
Gln>Glu	C>G	90%/10%	81%/19%	13
Arg>Ser	C>A	50%/50%	60%/40%	13
Ala>Ser	G>T	90%/10%	72%/28%	13
Ile>Leu	A>C	90%/10%	81%/19%	13
Ser>Ala	T>G	90%/10%	100%/0%	13
Glu>Asp/Ser	G>C,	50%/50%	60%/40%	13
Phe>Tyr	TTC>TAC	50%/50%	72%/28%	13
Gln>His	G>C	50%/50%	45%/55%	13
Gln>Gly	AGG>GGT	50%/50%	45%/55%	13

Functional Analysis

Four clones were randomly picked and grown in small scale expression cultures. Depending on the expression protocol periplasmic extract and supernatant were used in indirect ELISA to confirm binding activity of the scFv´s to the SAI/II antigen.



Conclusions and Future Perspectives

The shown data successfully prove the concept of creating a CDR grafted framework library for humanization of a murine antibody, by design, assembly, cloning and characterization of a hybrid humanizedVL - mouseVH library.

In the following the humanized VH library will be assembled and joined with the VL library to select humanized scFv´s with optimal binding characteristics and a high number of human residues. Biosassays will follow to show the activity and immunogenic potential. of the humanized molecules