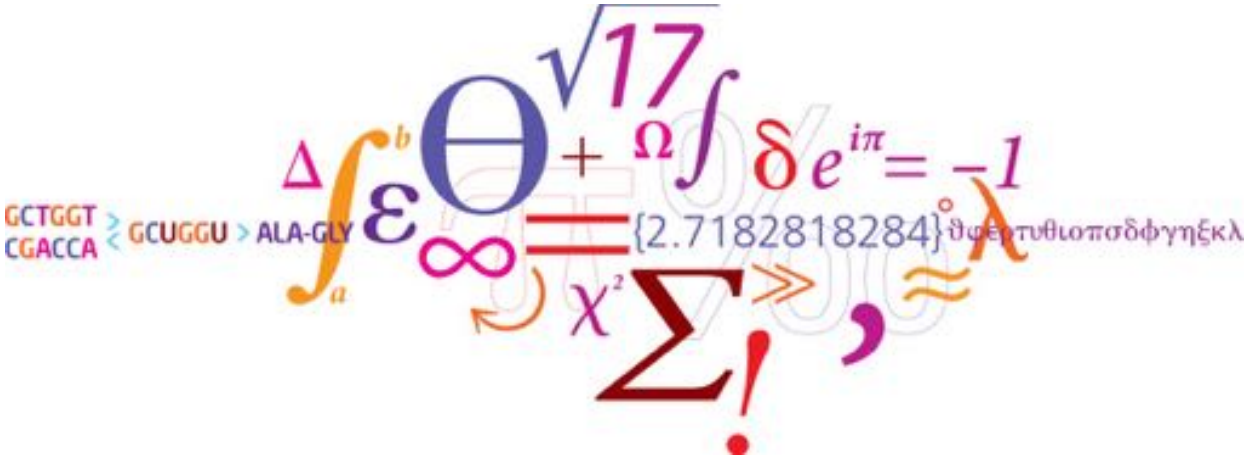
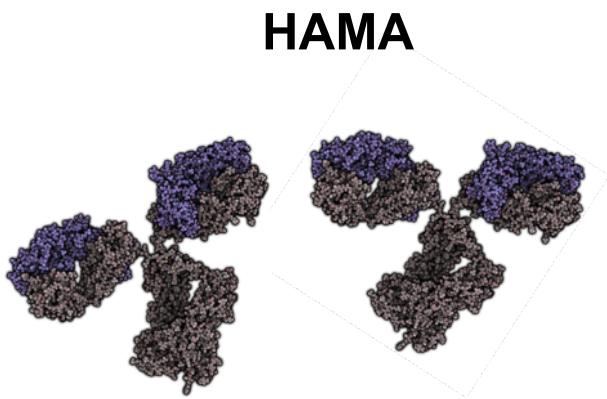
Antibody Humanization

Paolo Marcatili



DTU Biosys
Department of Systems Biology

Murine monoclonal Abs

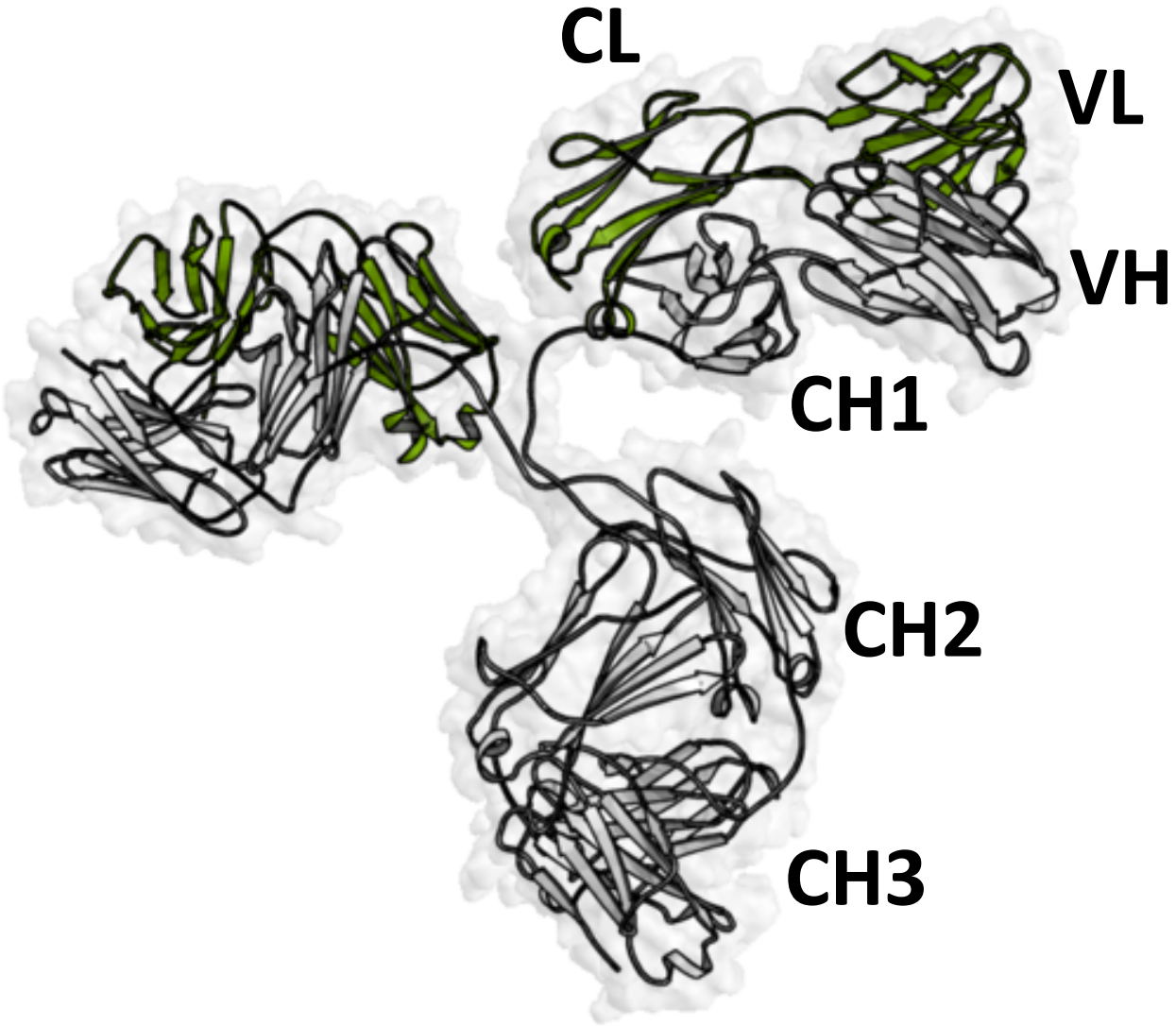


HUMANIZATION

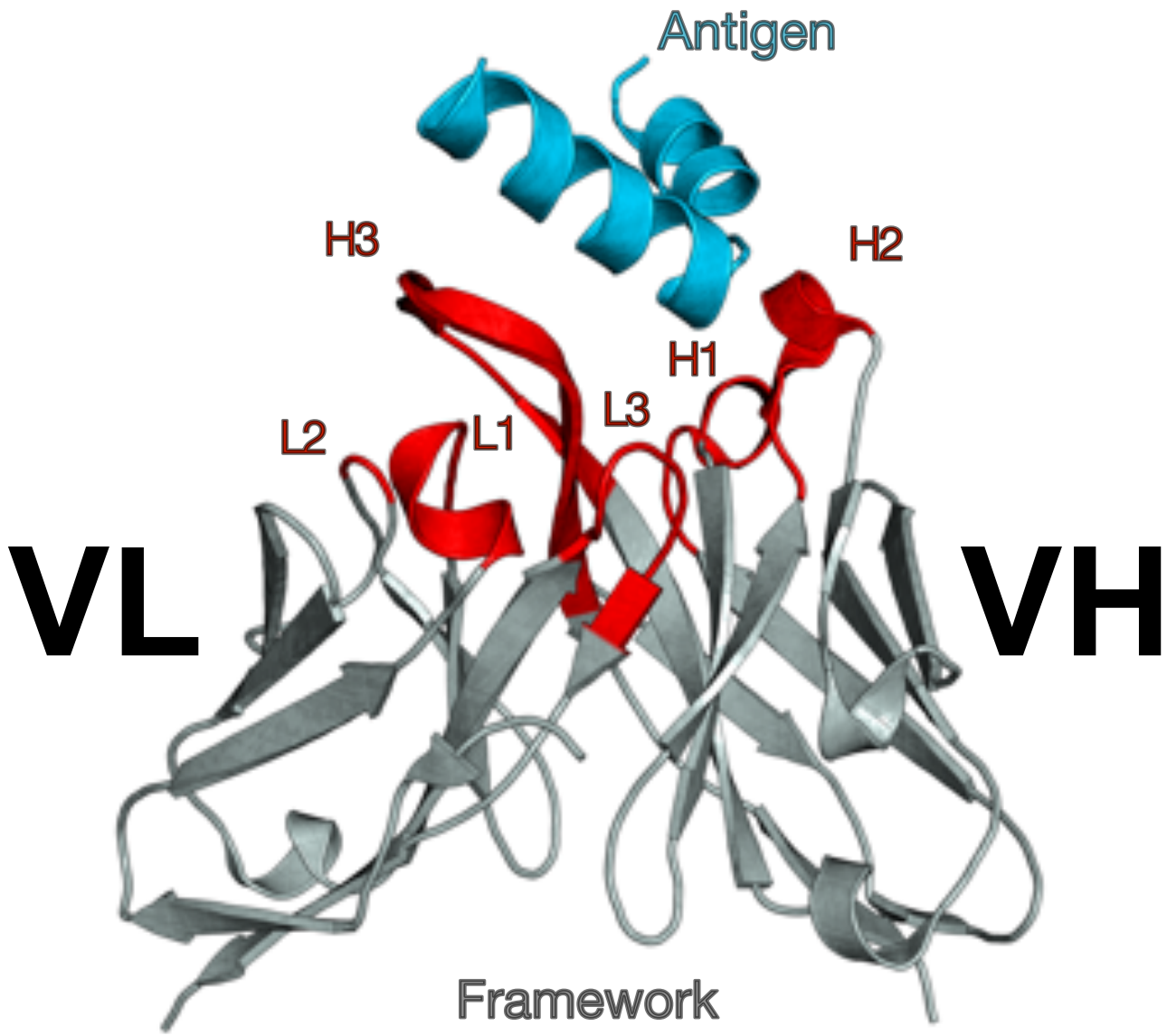
Rationale

We can engineer the antibody so that it "looks" human.

Antibodies are modular



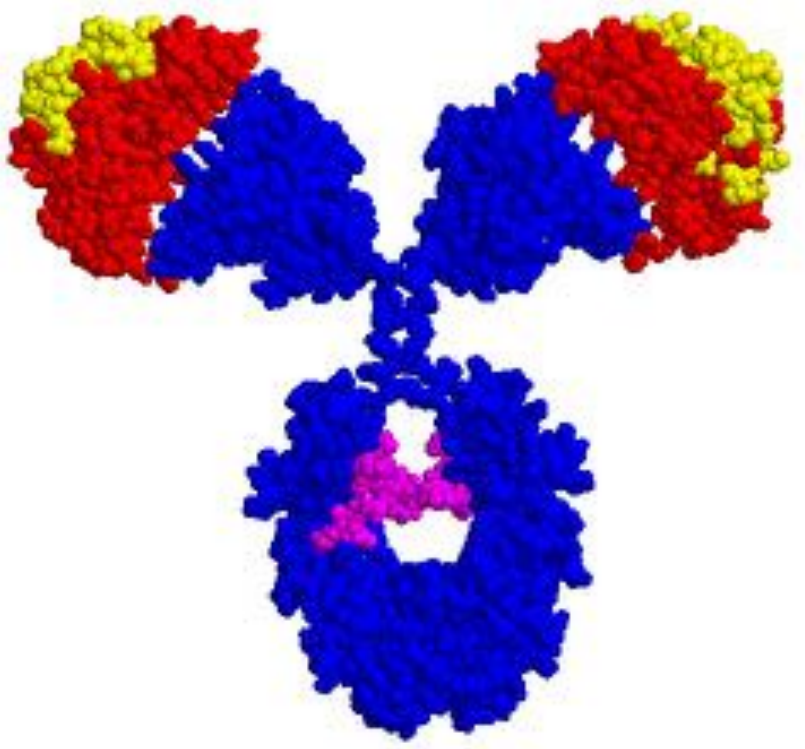
ABS



Chimeric antibody

Human Constant domain

Murine variable domains

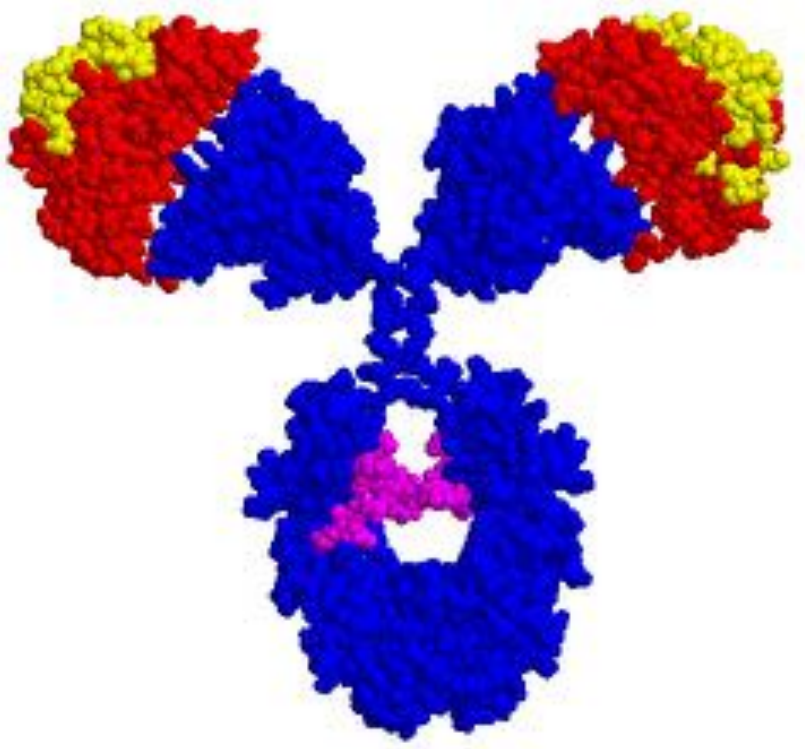


Chimeric antibody

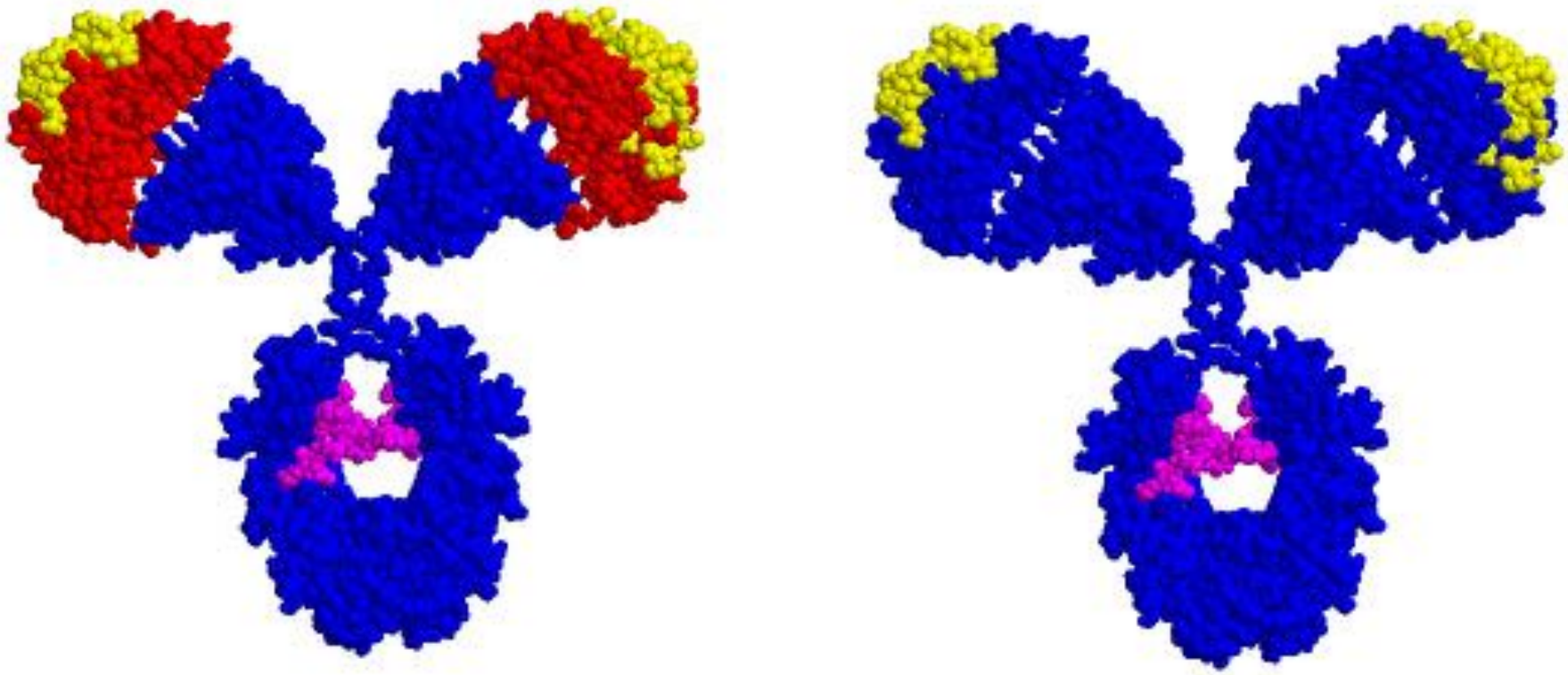
Human Constant domain

Murine variable domains

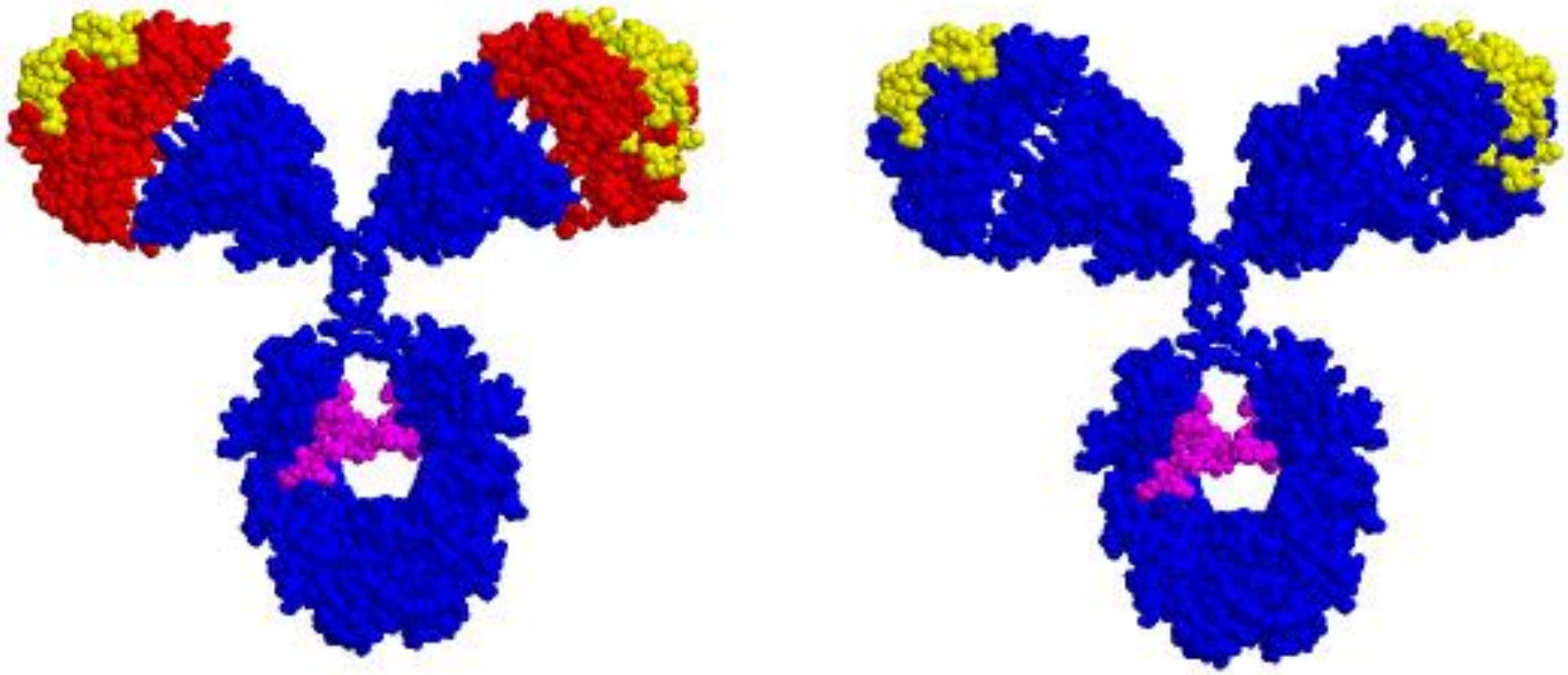
Still antigenic!



Chimeric vs humanised



Chimeric vs humanised



How much can we humanise without loosing affinity?

Humanization strategies

CDR grafting: Use only the murine CDRS

SDR grafting: CDR + structurally important residues (Canonical Structures)

Resurfacing: Change all surface residues (apart to CDR) to human

Superhumanization: Check for each segment in the Ab and add mutation to maximize local similarity to the human

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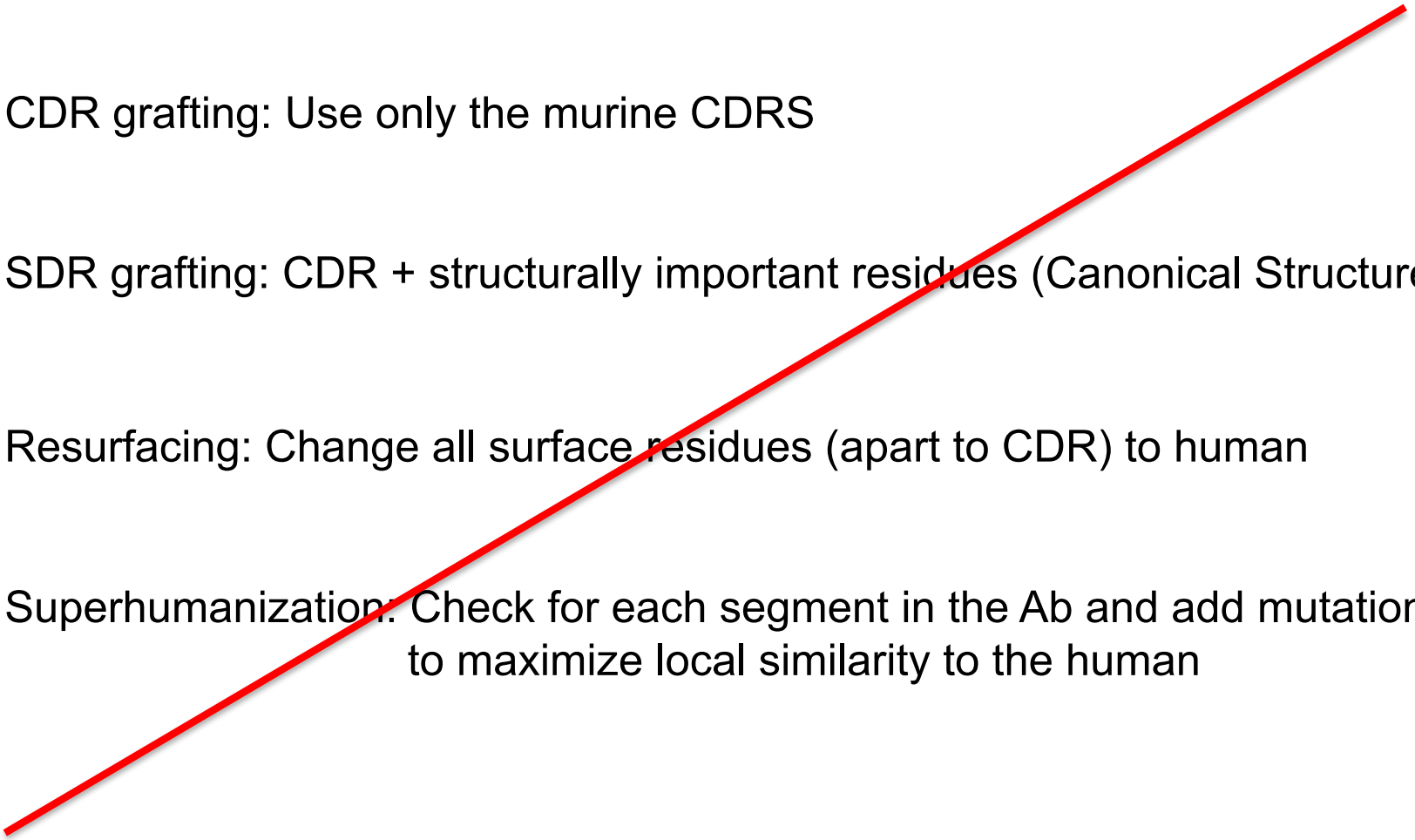


Humanization strategies

CDR grafting: Use only the murine CDRS

usually the antibody loses affinity

SDR grafting: CDR + structurally important residues (Canonical Structures)

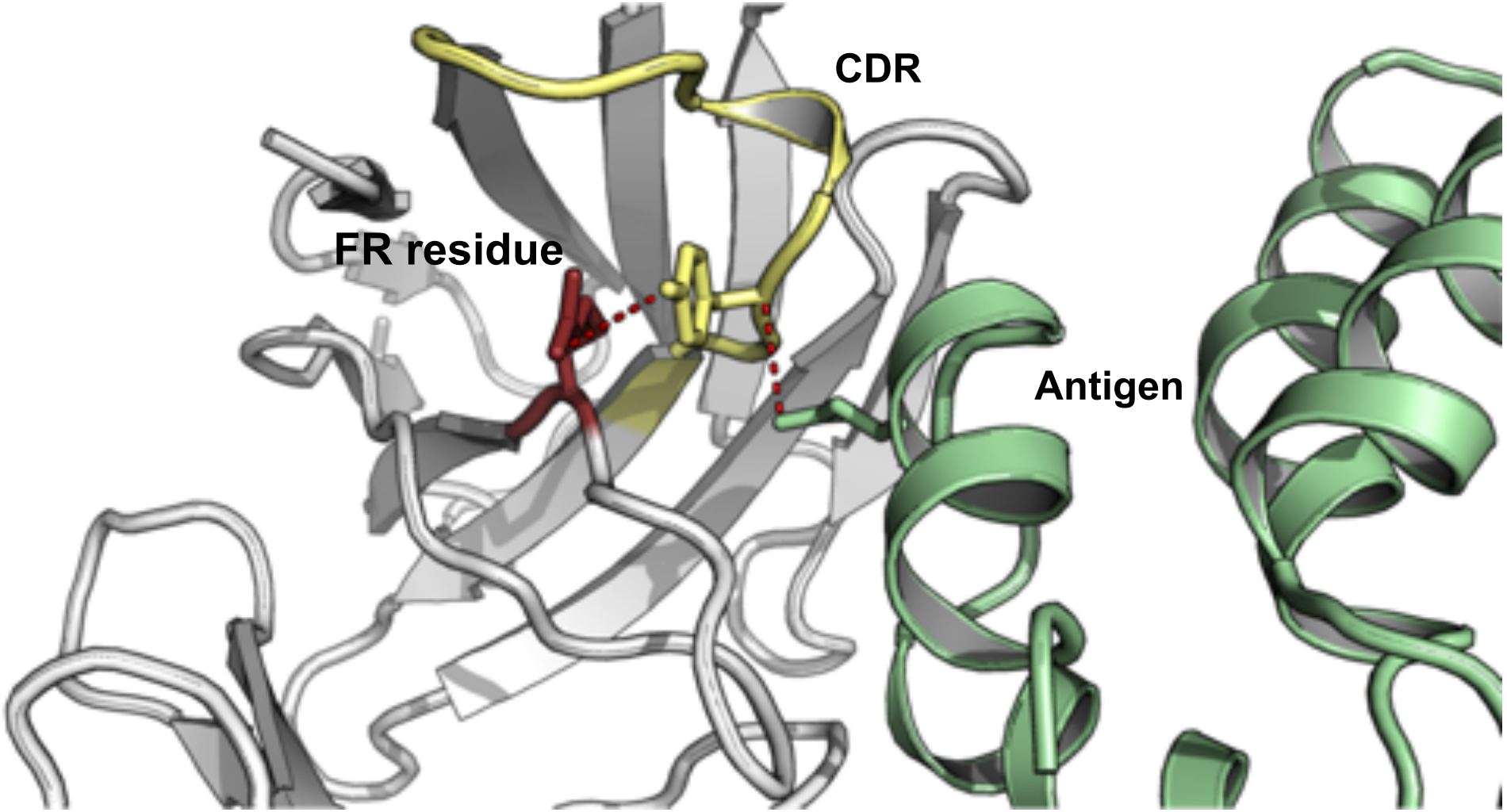
often binding is poor

Antibody Paratope

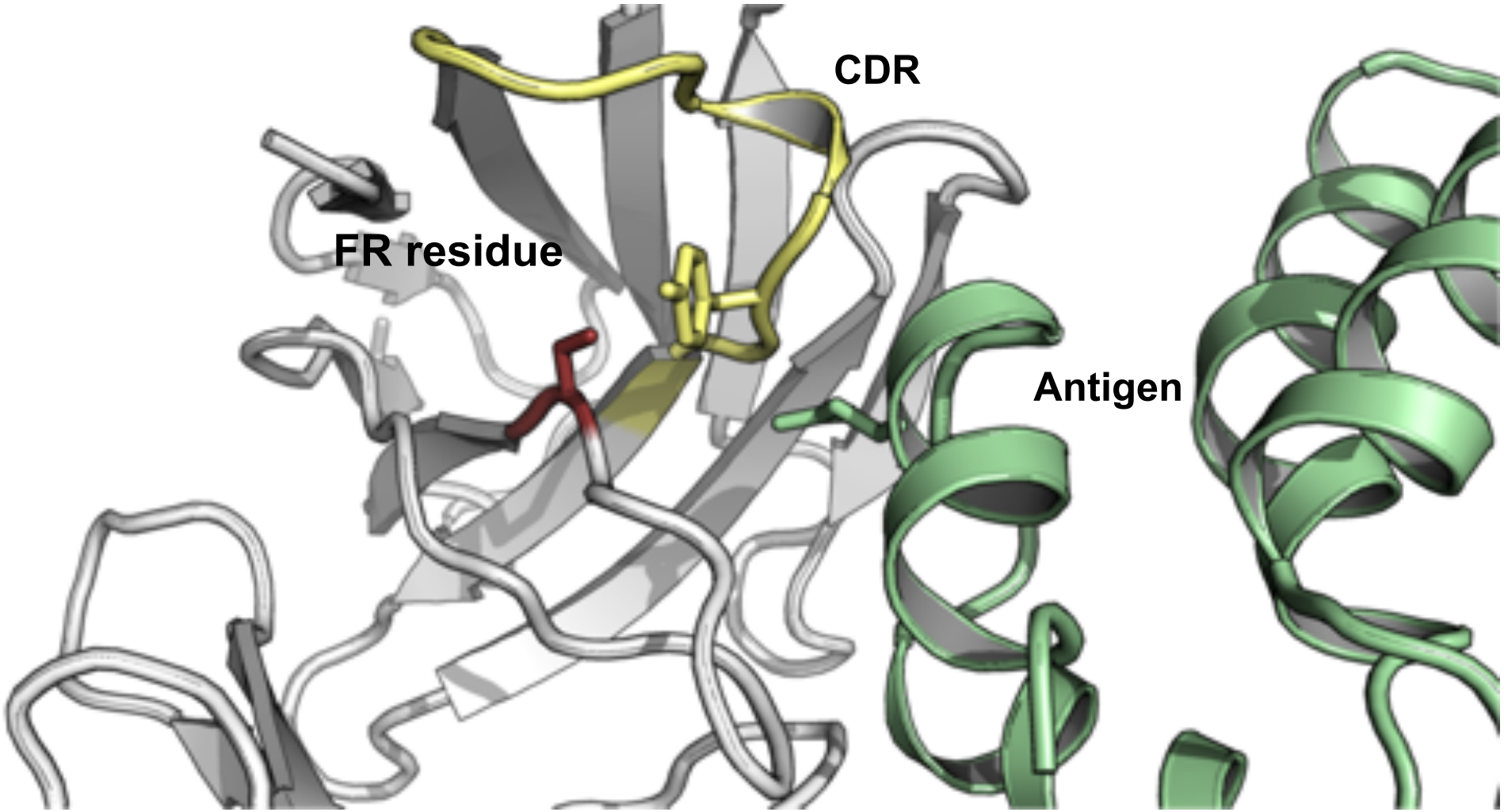
The binding probability of each residue depends on all the environment.

Even distant residues might change the binding mode

Framework residues affect antigen binding (Mouse)

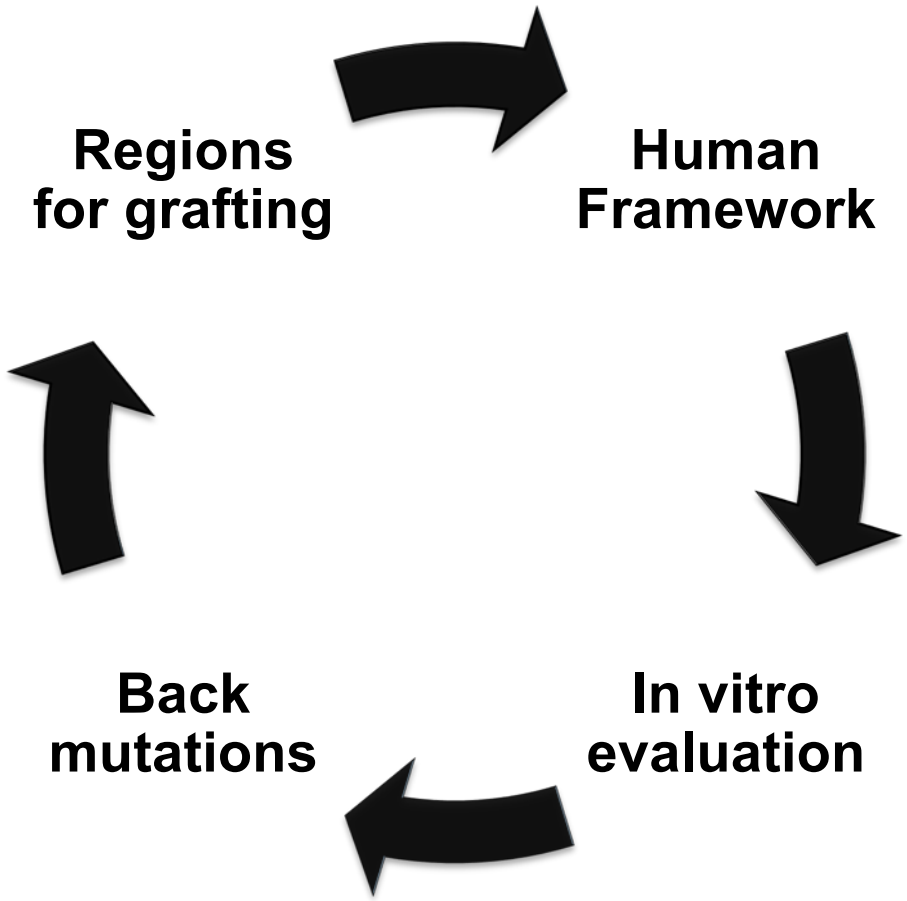


Framework residues affect antigen binding (Mouse)

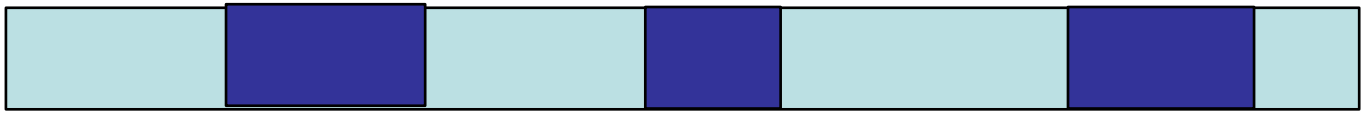


Humanization process

"*Trial and error cycle*"

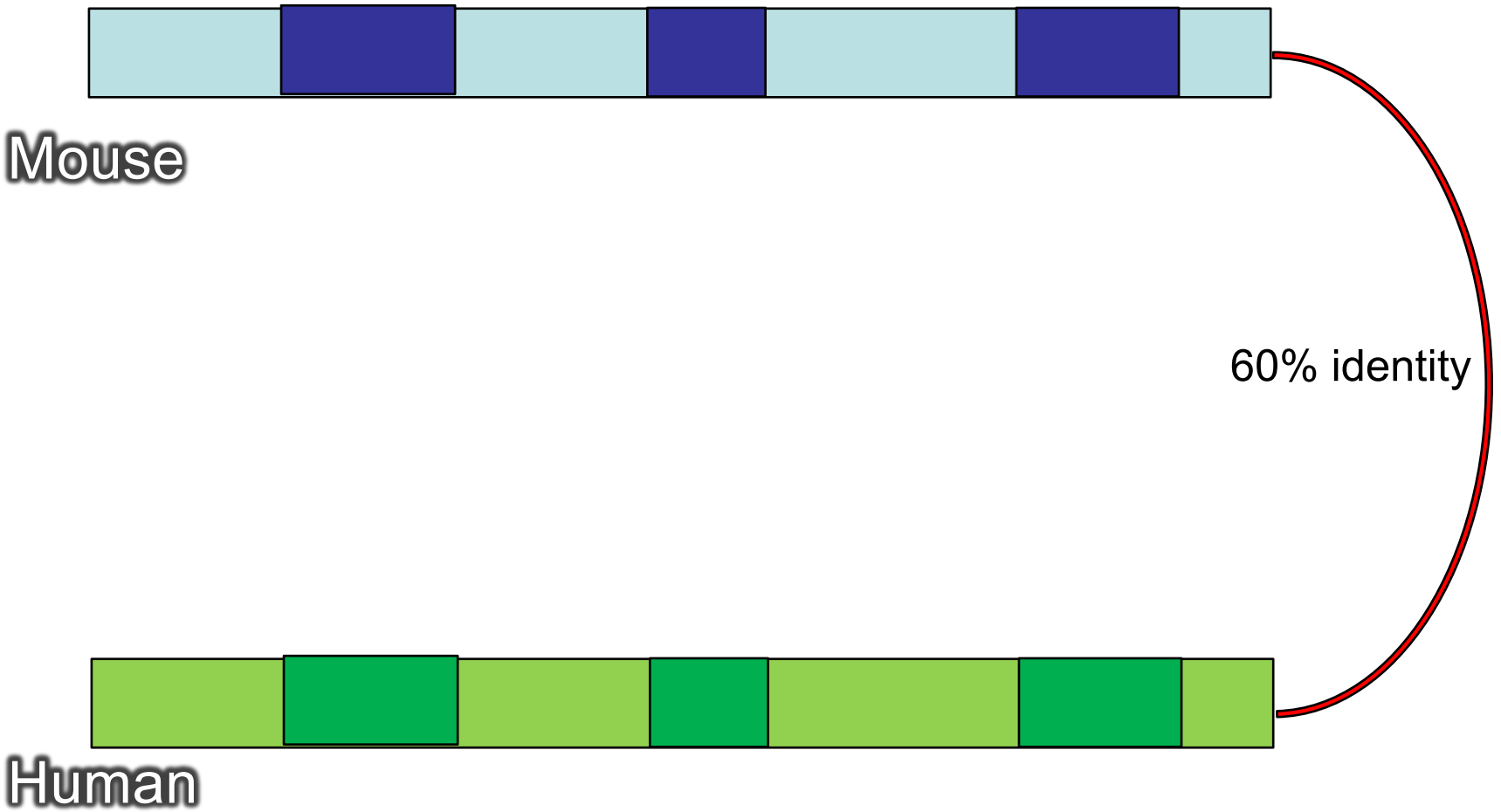


Rationale

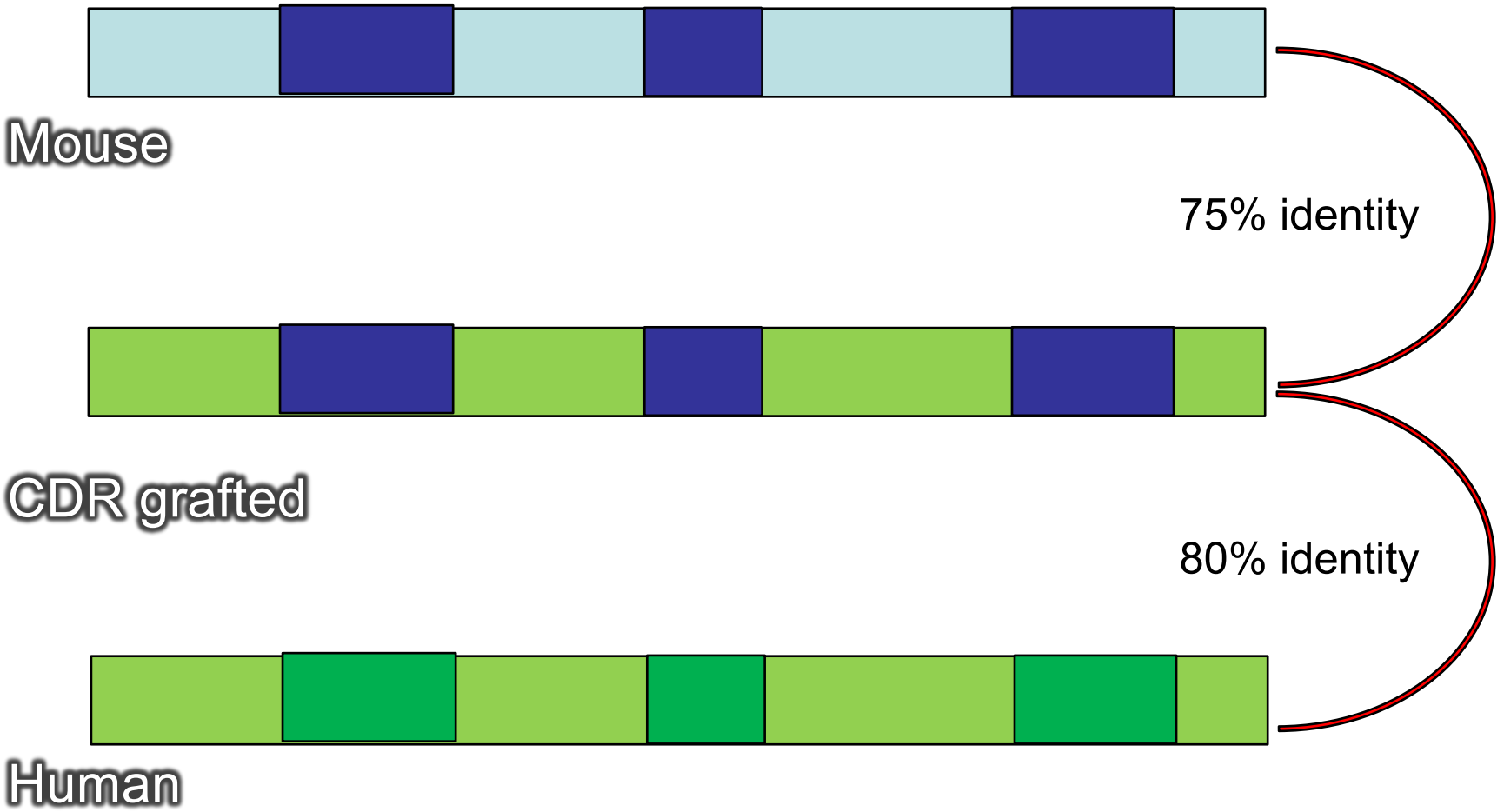


Mouse

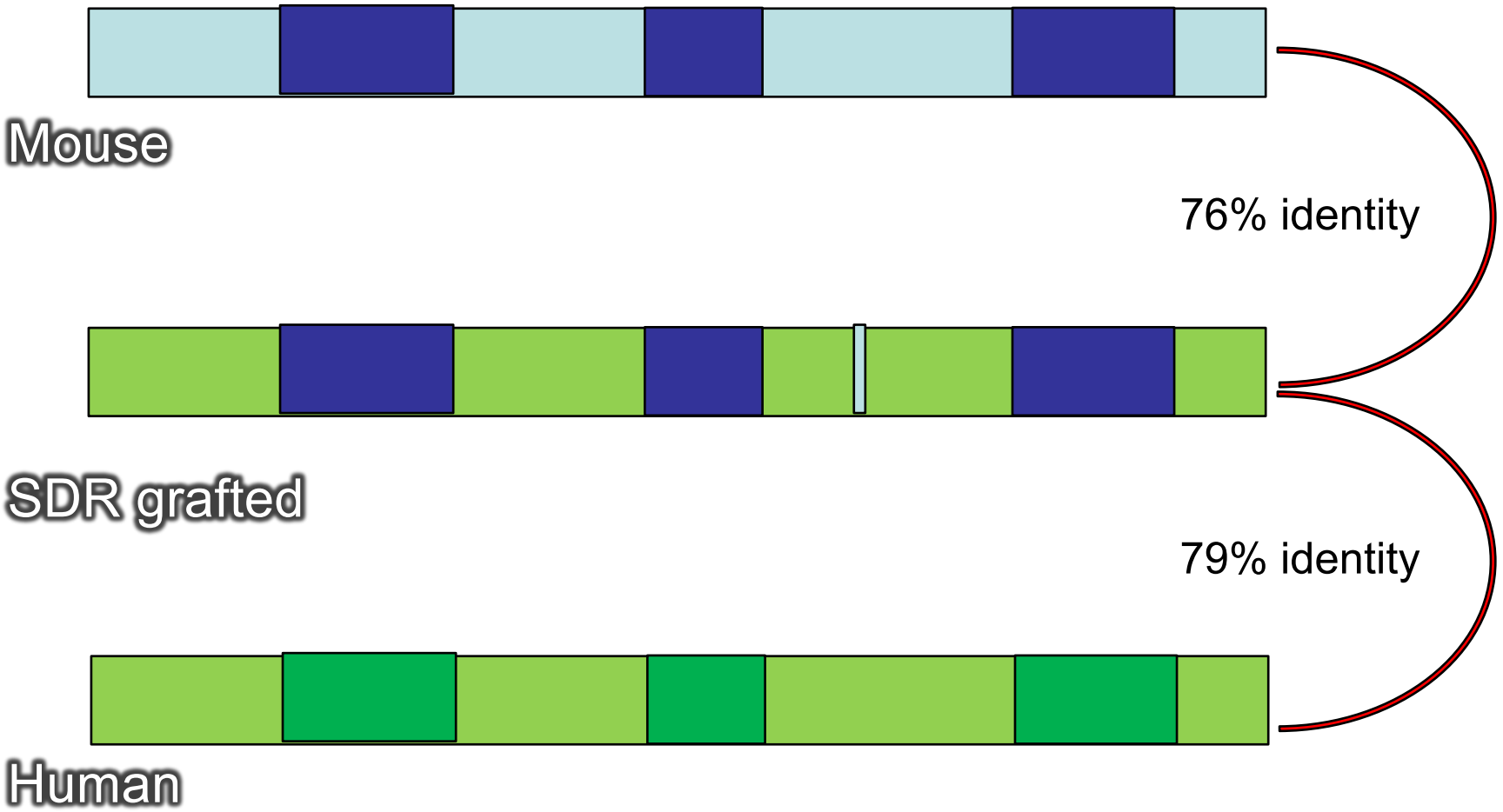
Rationale



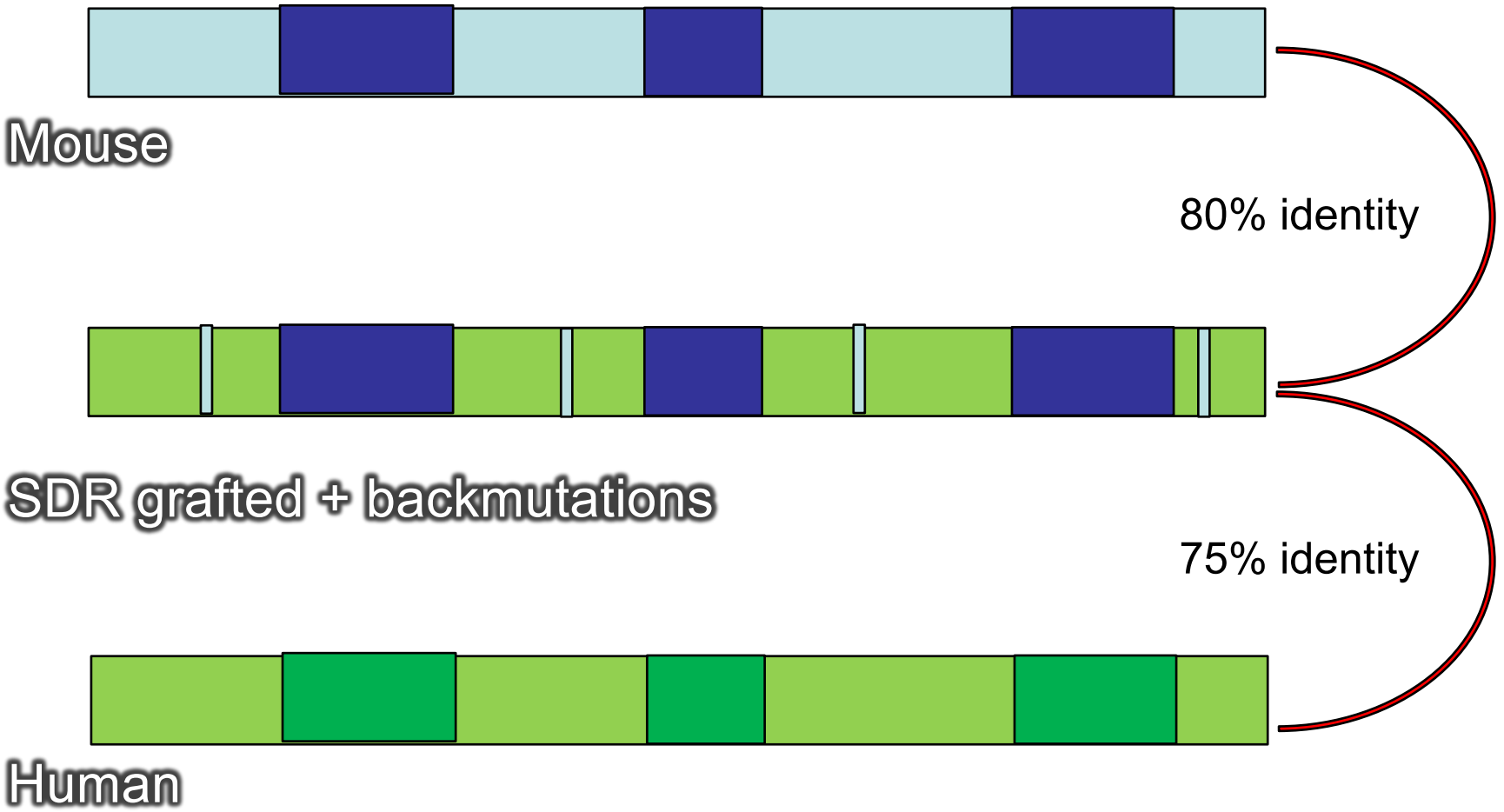
Rationale



Rationale



Rationale


Mouse
80% identity
SDR grafted + backmutations
75% identity
Human

Basic Protocol

1) Identify Murine germlines
2) Identify the murine CDRs (IMGT, Kabat, etc.)
3) Identify Human acceptor
    i- germlines
    ii- complete antibody
4) Graft the CDR
5) Identify all backmutations
6) Select Backmutations to include
7) Test production, affinity, immunogenicity etc.
8) If results are not good, go to 6

Backmutations

How to priorities backmutations:

- Are important for the structure (Vernier zone, L/H interface)
- Modify the charge
- Prevent clashes and cavities
- Do not increase the risk of immunogenicity
- Improve expression (literature)
- No additional PTMs or digestion sites

Conclusion

Antibody humanization is needed for any Ab drug

It is still a "trial and error" procedure

non CDR residues can greatly affect the binding

Structural analysis is extremely important