Biotechnological approach to a biopharmaceutical product development

A biotechnologist point of view

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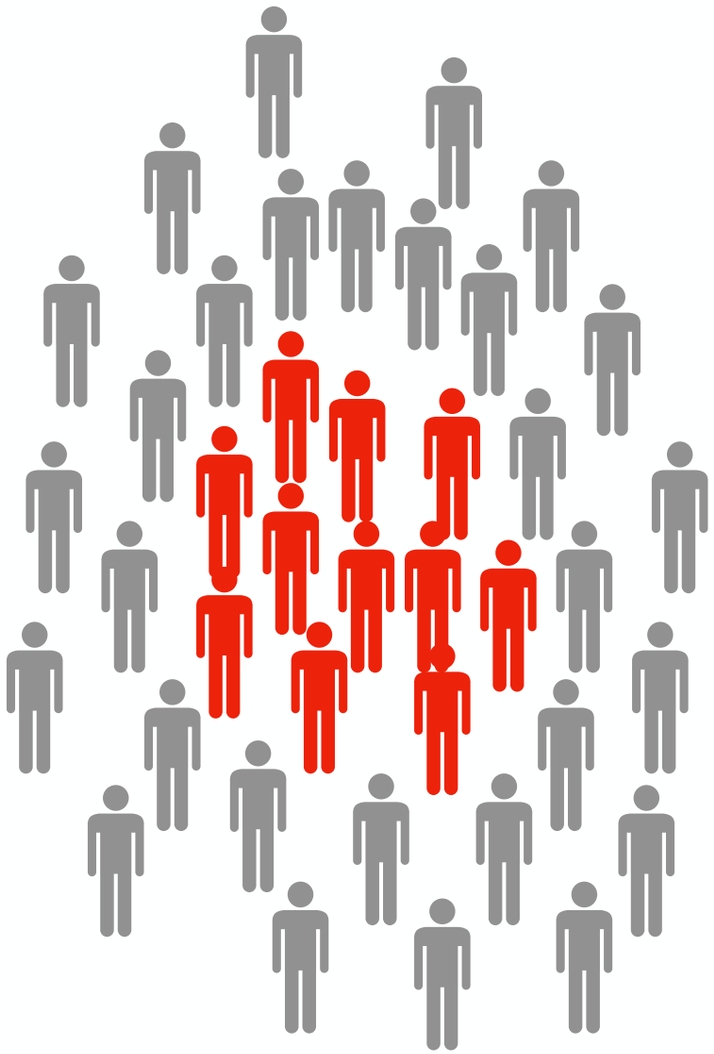
Contents

1. Monoclonal antibody (mAb)

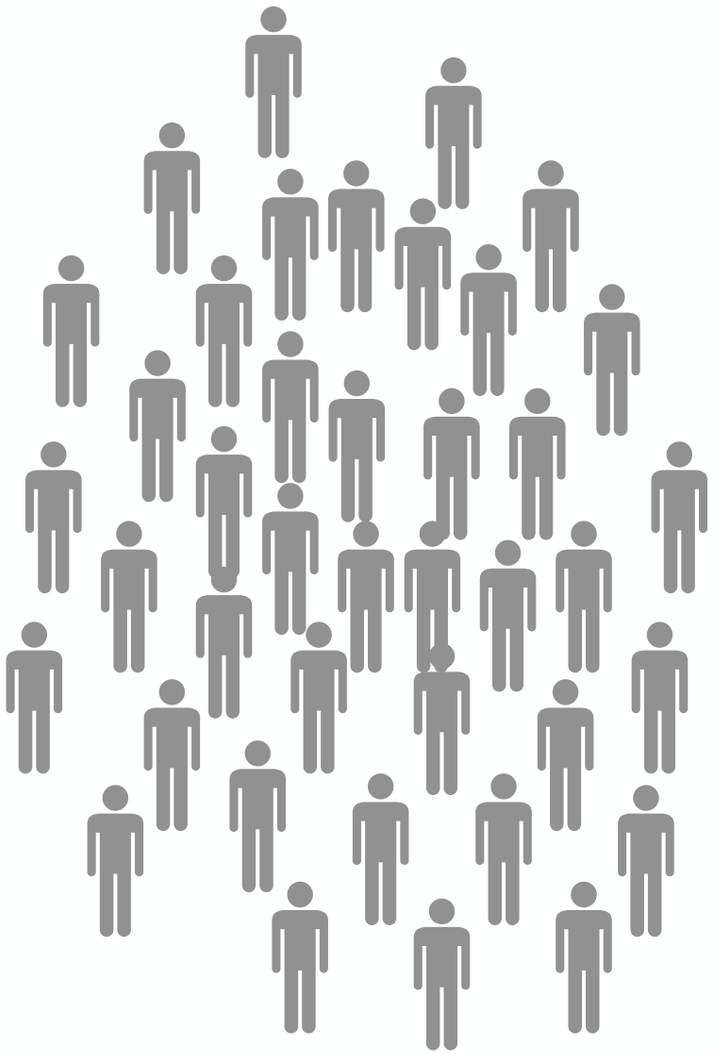
CoV2 eradication strategies

preventive & curative



prevent

vaccine

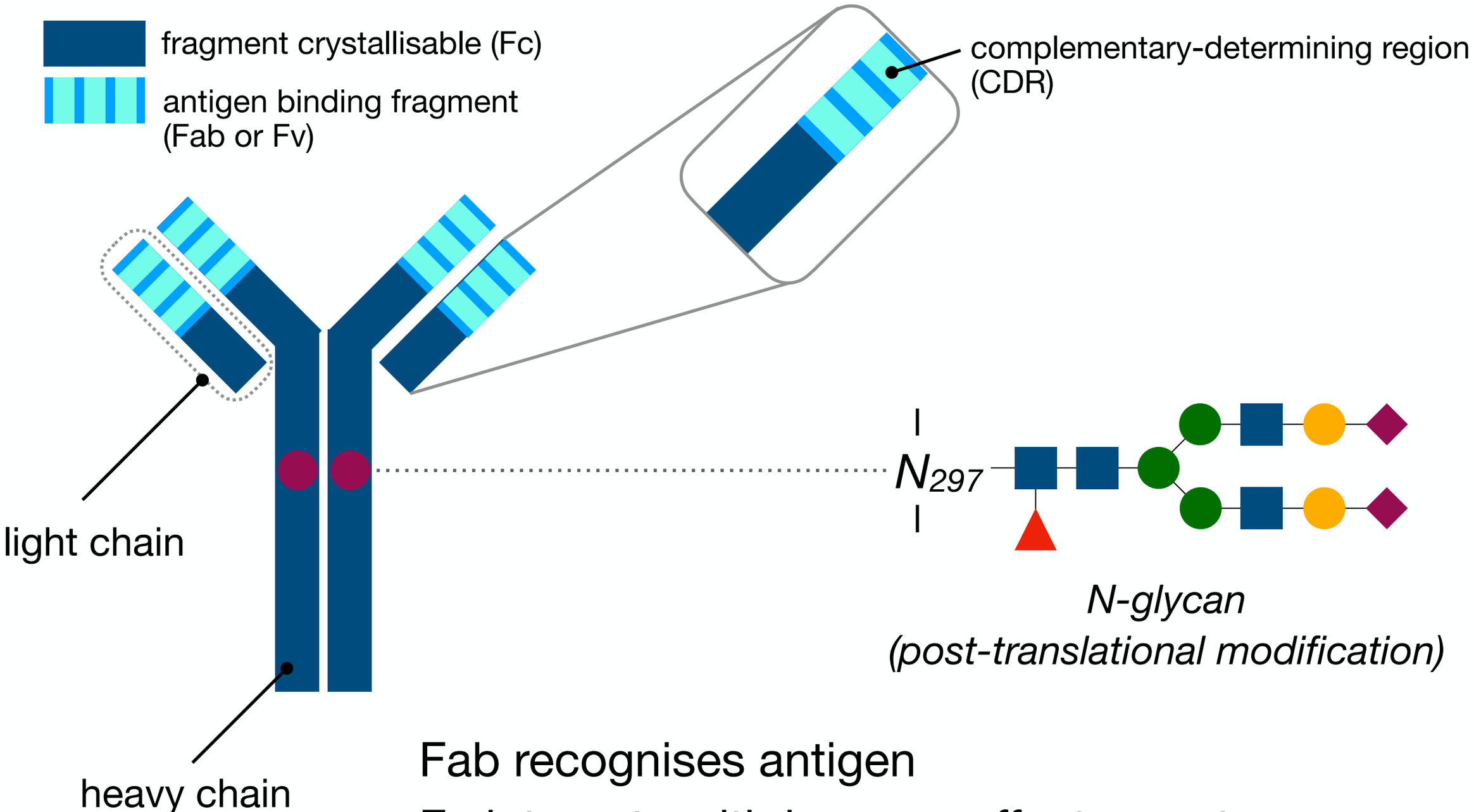


cure

monoclonal antibody (mAb)

Monoclonal antibody

tools to combat massive number of antigens



Fab recognises antigen

Fc interacts with immune effector system

Therapeutic mAbs development

historical overview

César Milstein & Georges Köhler expressed mAb using **hybridoma technology**

1975

First **clinical use** of murine hybridoma-produced mAb

1986

Murine
(-omab)

Rituximab, a **chimeric mAb** approved for clinical use

1997

Chimeric
(-ximab)

Trastuzumab, a **humanised mAb** approved for clinical use

1998

Humanised
(-zumab)

Adalimumab, a **human mAb** introduced for clinical use

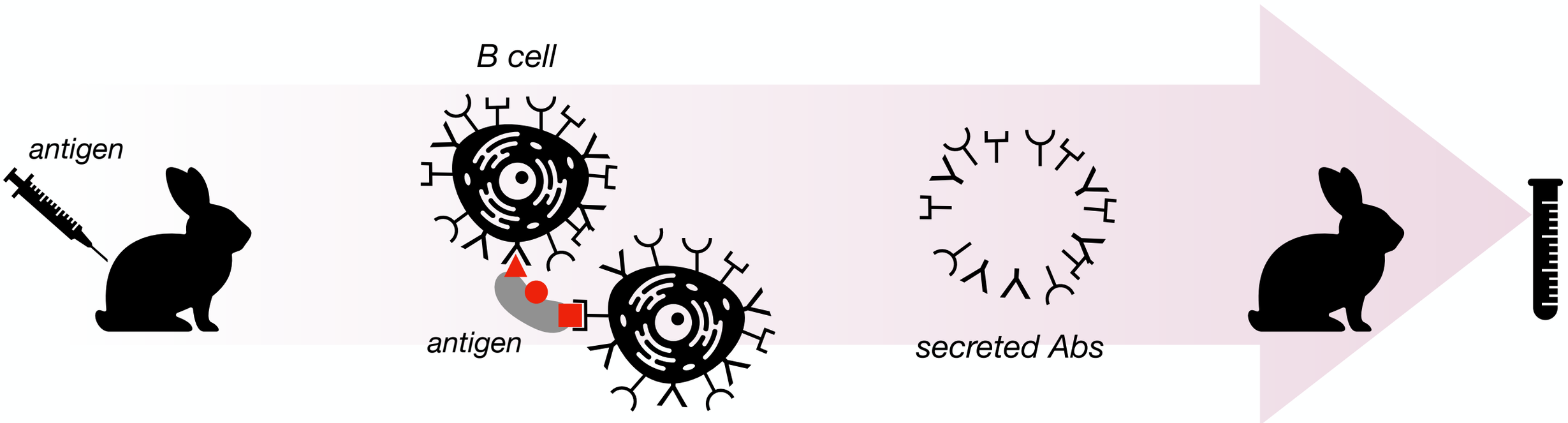
2002

Human
(-umab)

Hybridoma technology

the foundation of therapeutic mAbs production

Past - polyclonal Abs (pAbs)

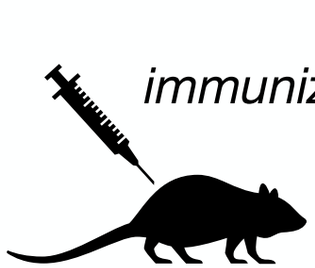


1. Safety
   - low specificity
   - induce HAMA response

2. Quantity
   - repeated animal use (mortal)
   - low yield

Hybridoma technology

the foundation of therapeutic mAbs production



immunization

B lymphocytes (Ab producer)

Myeloma (immortal)

X

fusion

HAT selection

ELISA screening & recloning

Immortalised B-cell (hybridoma)

Mass culture

monoclonal & immortalised

high specificity

HAMA response

human Fc receptor insensitive

infinite

murine mAbs

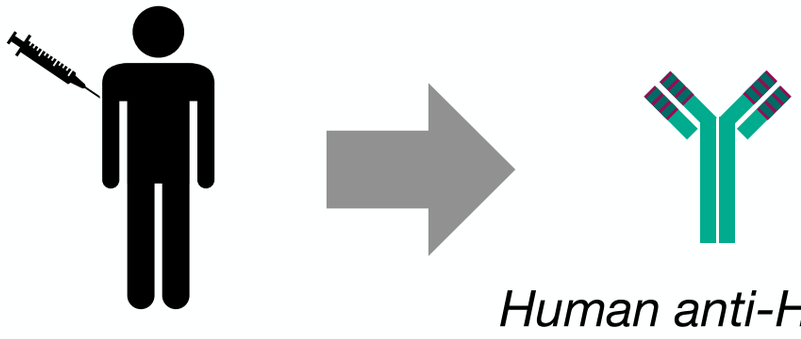
Why murine mAB induces HAMA response?

maybe because it’s origin

Second generation mAbs

the chimeric and the humanised

Attempts to eliminate HAMA response & increase human Fc receptor sensitivity



HBV vaccine (rHBsAg protein)

Human anti-HBsAg

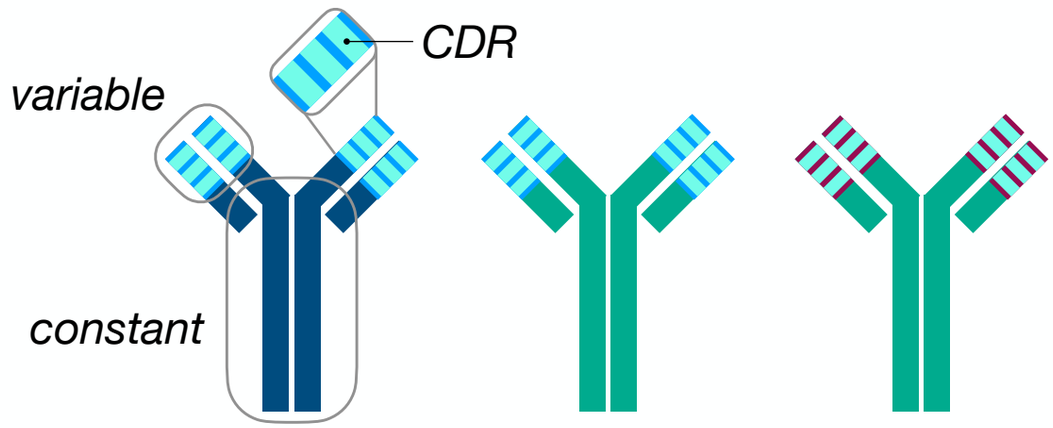
from human, for human

safe, human Fc receptor-sensitive

how to create chimeric & humanised mAb?

“Engineer the murine part of the mAb to be as much human part as possible”

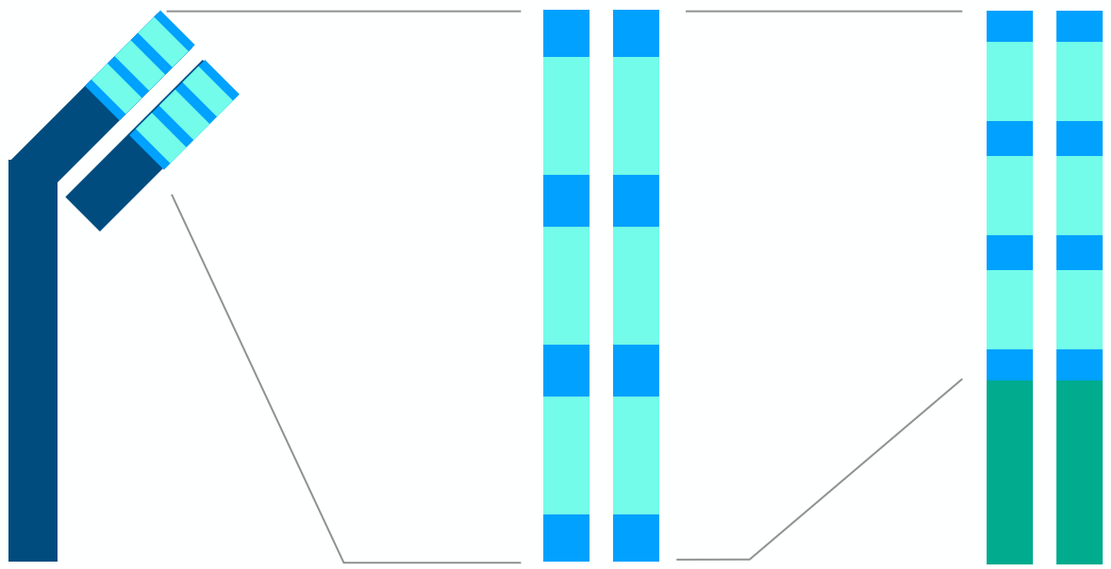
humanisation efforts



variable	murine	murine	human
CDR	murine	murine	murine
constant	murine	human	human
mAb type	**murine**	**chimeric**	**humanised**

Second generation mAbs

the chimeric and the humanised

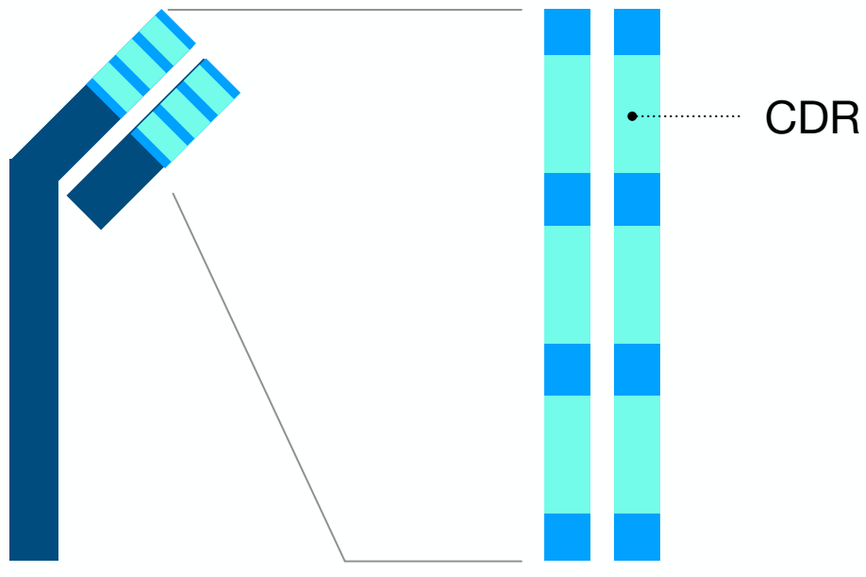


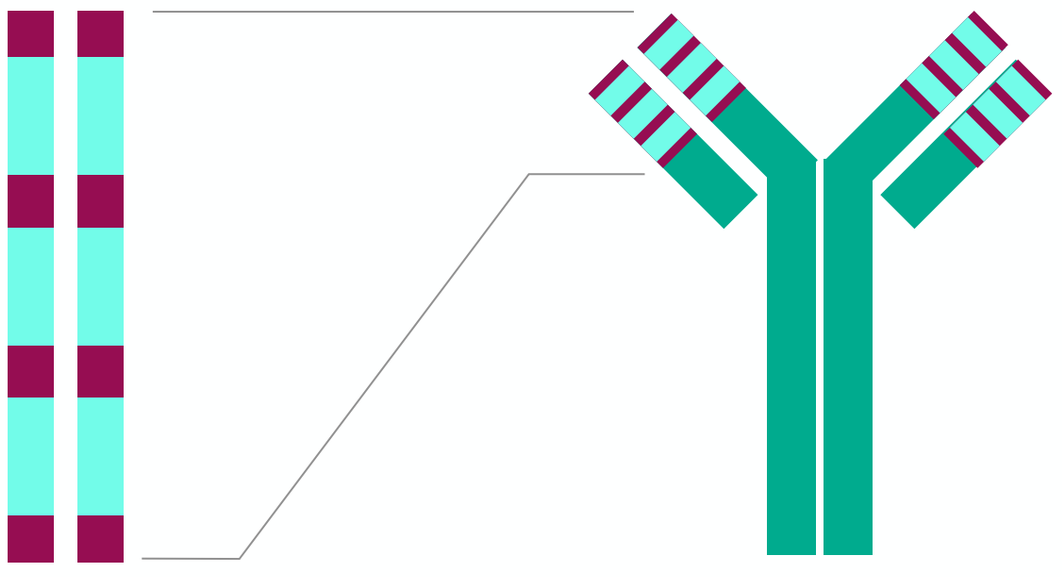
murine mAb

murine Fv

clone murine Fv to human Fc

Chimeric mAb





murine mAb

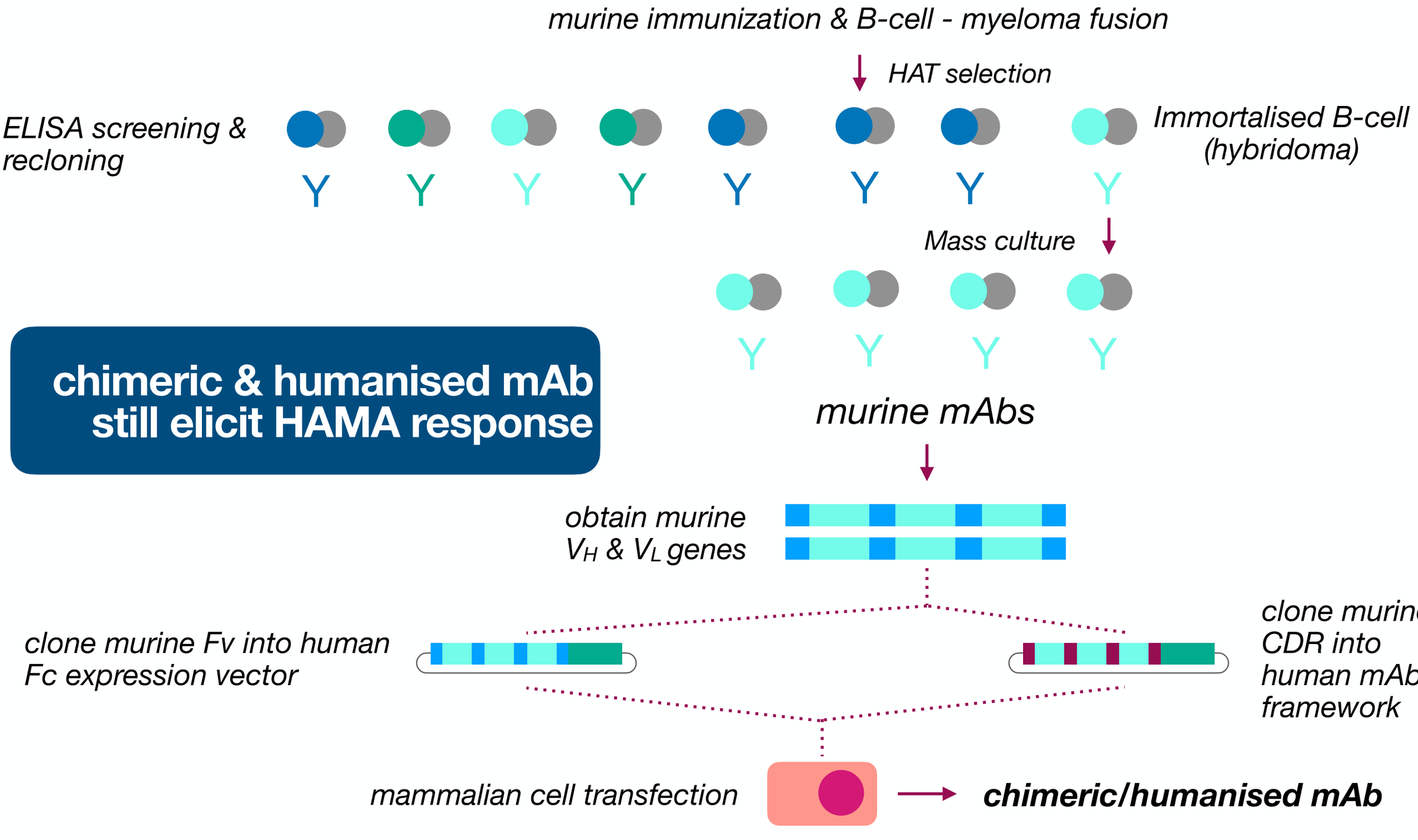
murine Fv

clone murine CDR to human frameworks

Humanised mAb

Second generation mAbs

the scheme



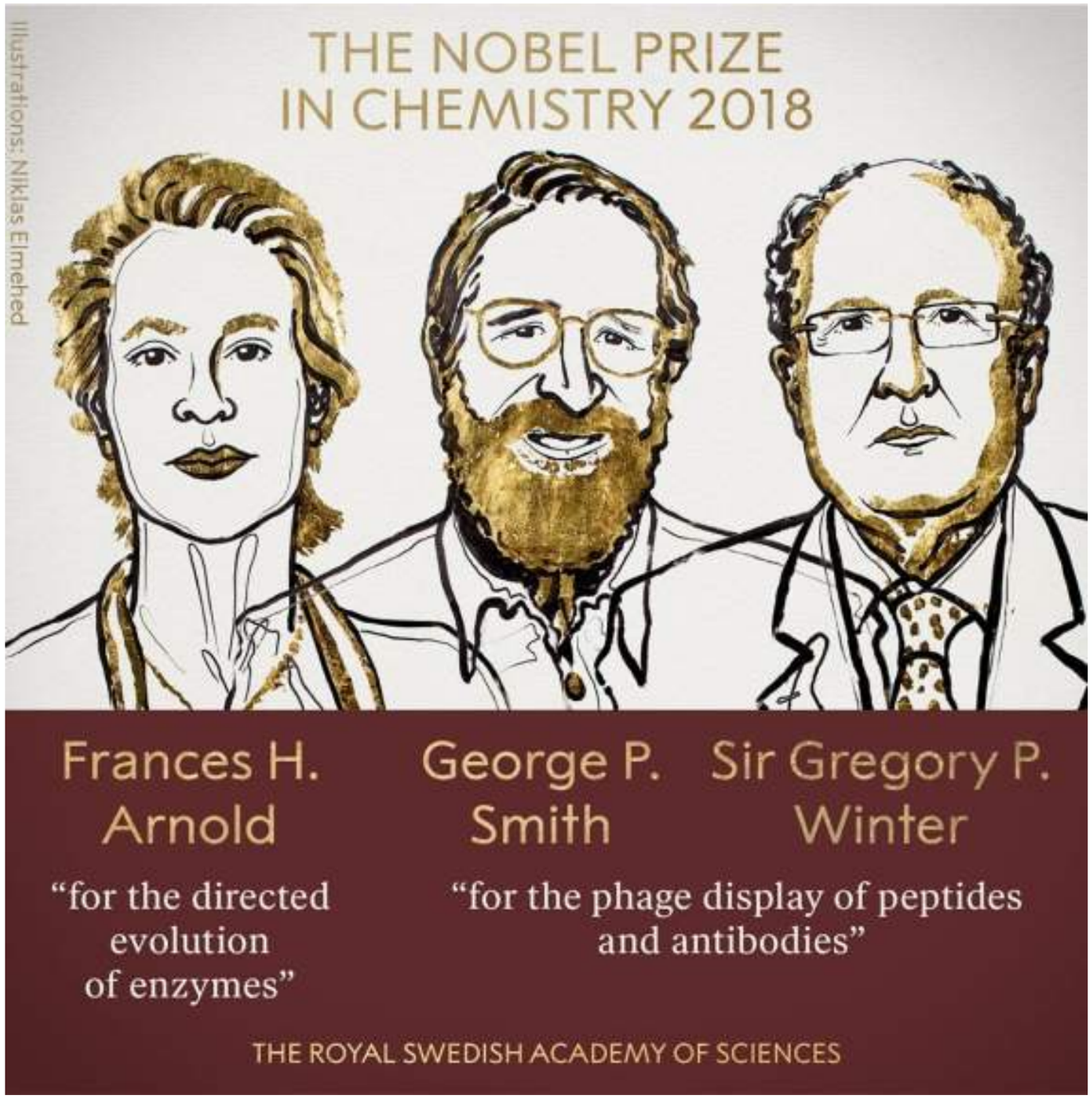
chimeric & humanised mAb still elicit HAMA response

Why human mAbs?

safety

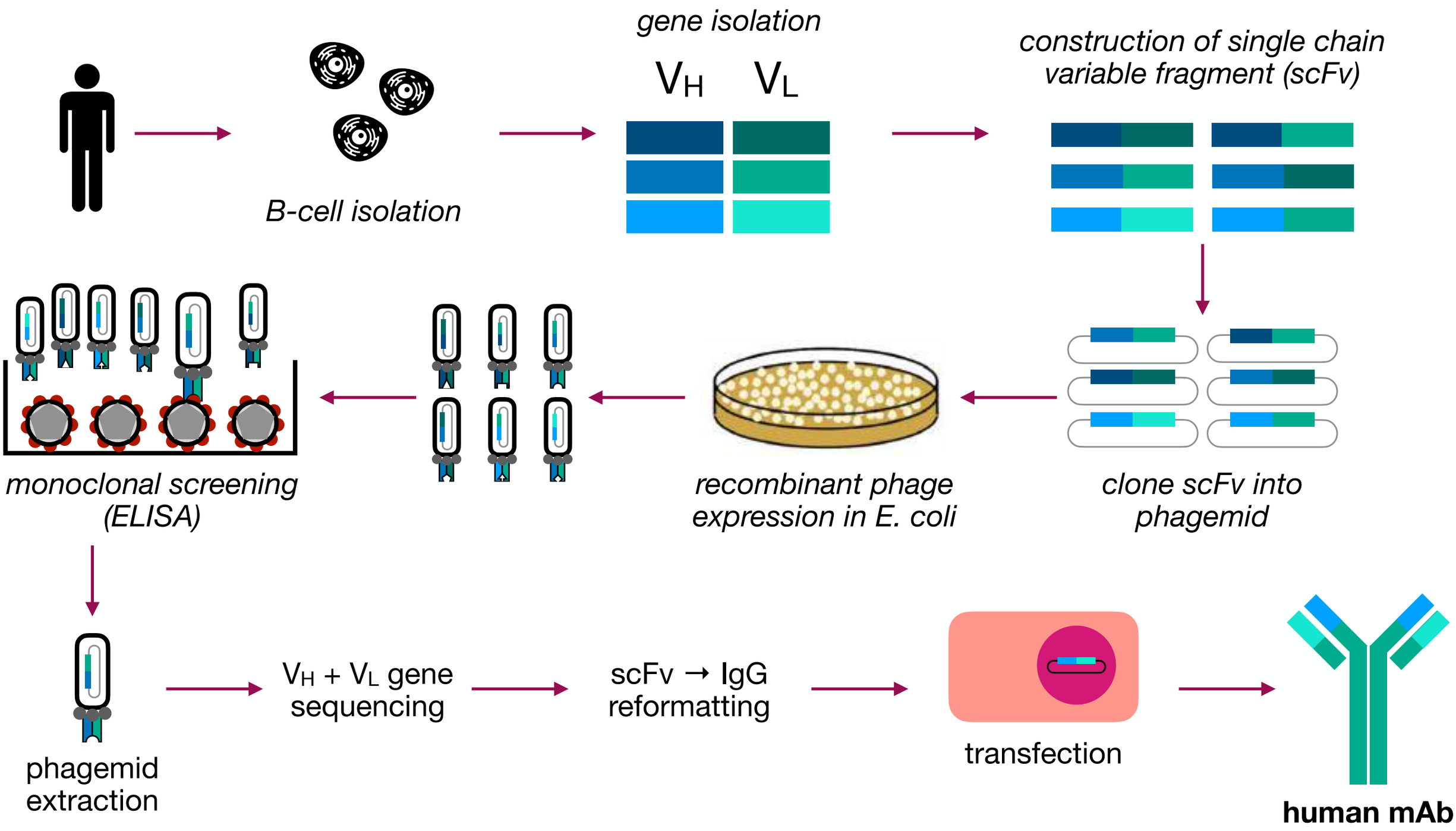
The biggest question

how to produce human mAb without using humans?



Third generation mAbs

“the fully-human” without using humans



3rd gen mAbs in relation to CoV2

Covid-19 survivors can help researchers

- Covid-19 survivors are critical in the development of anti-CoV2 mAb
- The soluble anti-CoV2 antibody is circulating in their blood-stream
- Their B cell is still expressing high amount of V_H and V_L genes responsible for CoV2 recognition
- Can ease researchers work to screen scFv, thus, minimise the time for optimum Fv development

Mini-summary

- Hybridoma technology is the foundation for modern mAb production
- More human sequence = safer
- In third generation mAb, Fv sequence was obtained from human, to be expressed in non-human production system
- Human protein can be produced in non-human production system (*e.g.* mammalian expression system)

2. Industrial perspective on the plant-based biopharmaceuticals

Monoclonal antibody is a glycoprotein

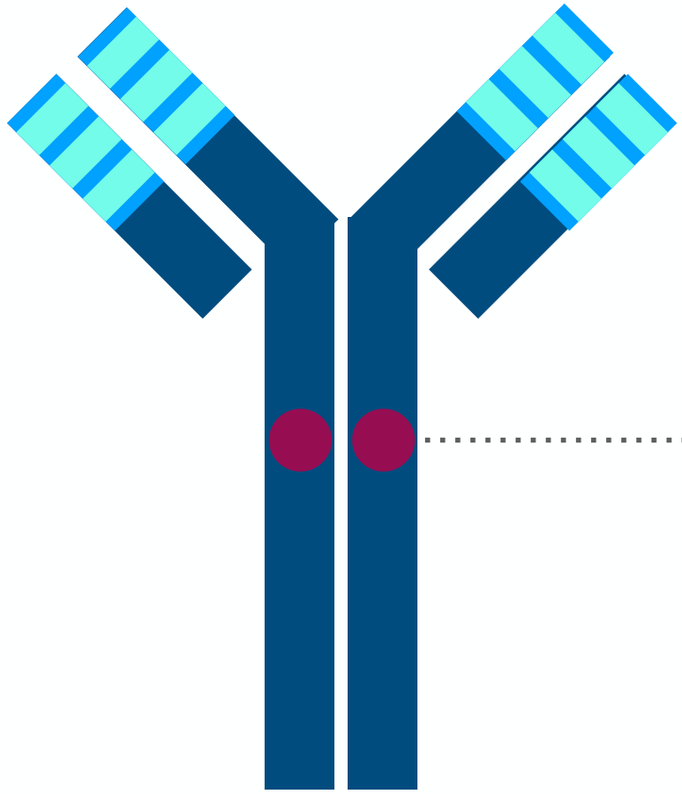
tools to combat massive number of antigens

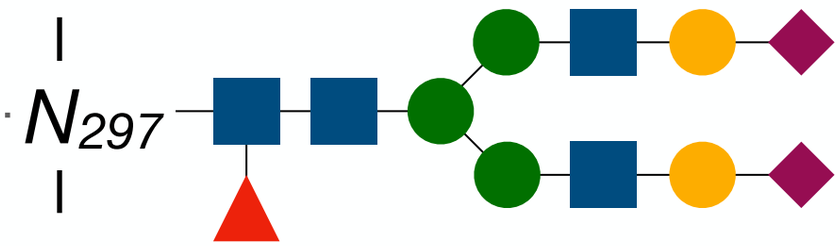
fragment crystallisable (Fc)

antigen binding fragment (Fab or Fv)

Fab recognises antigen

Fc interacts with immune effector system

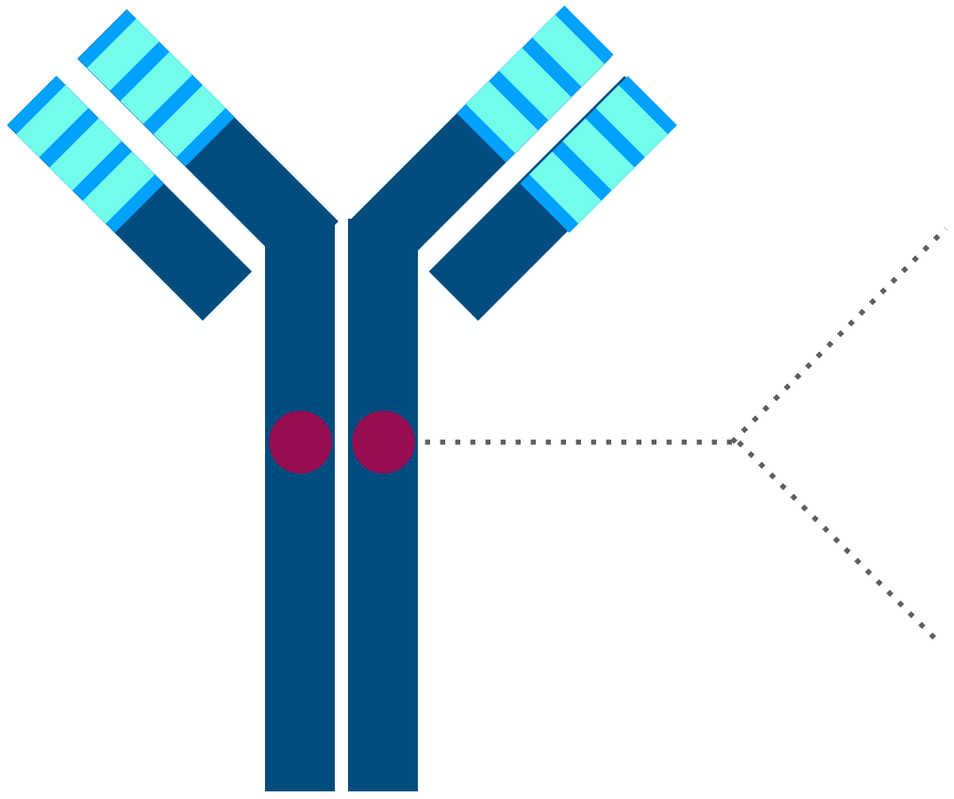


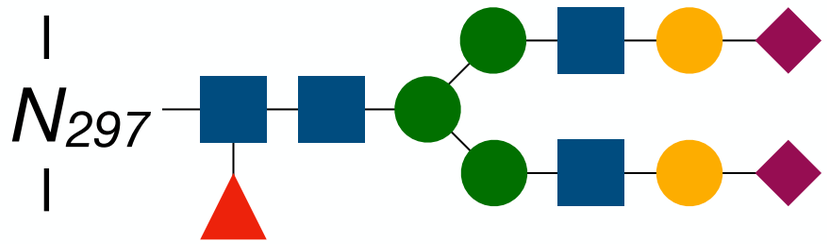


N-glycan
(post-translational modification)

N-glycan role in immune system

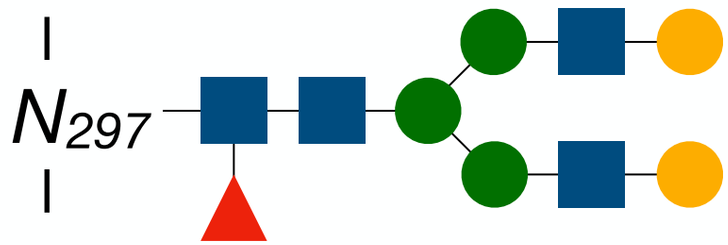
N-glycosylated Fc modulates antibody activity





Sialylated N-glycan

do not attack



Asialo N-glycan

attack!

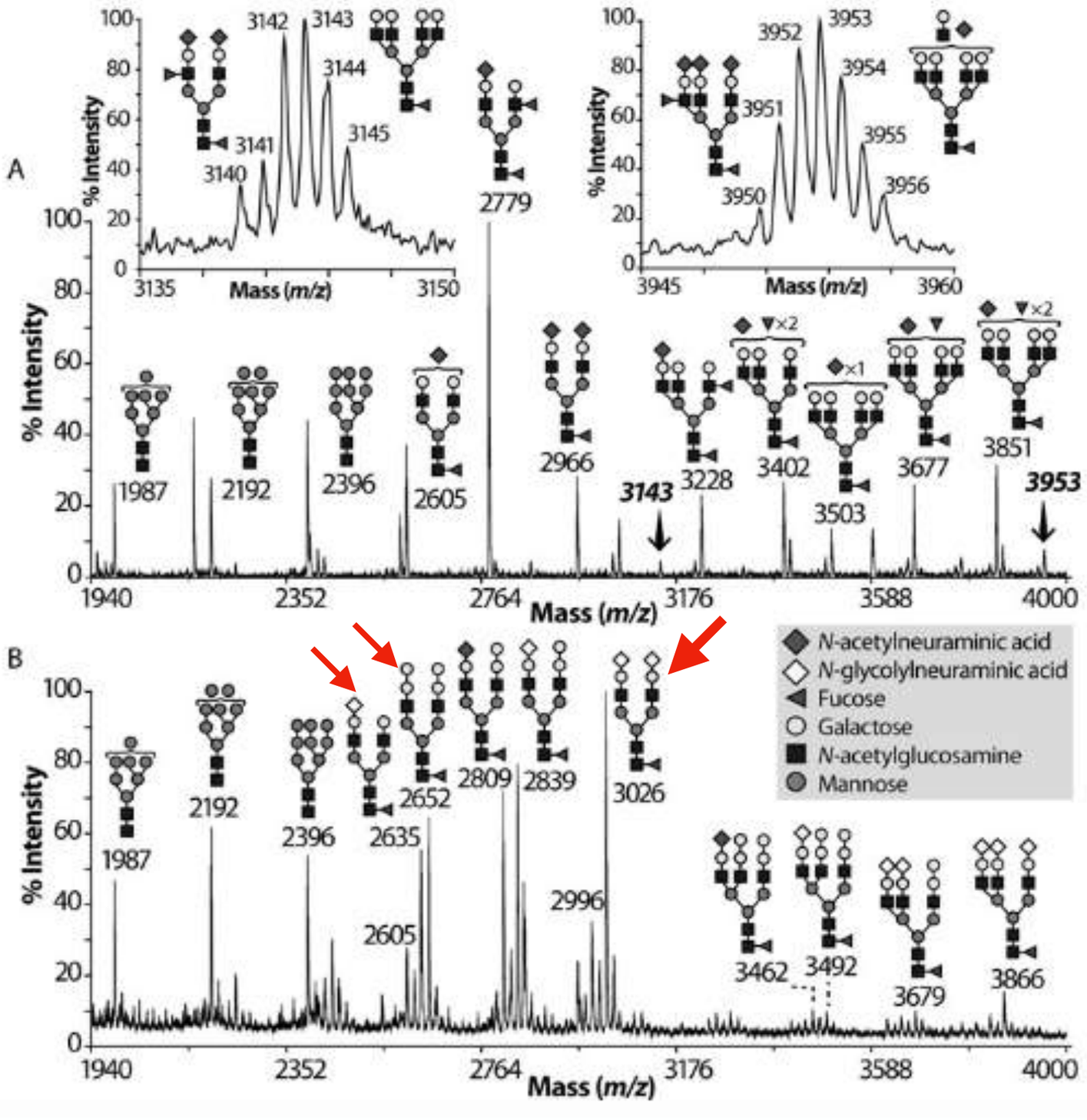
◆ Sia (NeuAc) ● Gal ■ GlcNAc ● Man ◀ Fuc

not only the protein sequence, *N*-glycan motifs may also play part in immune responses

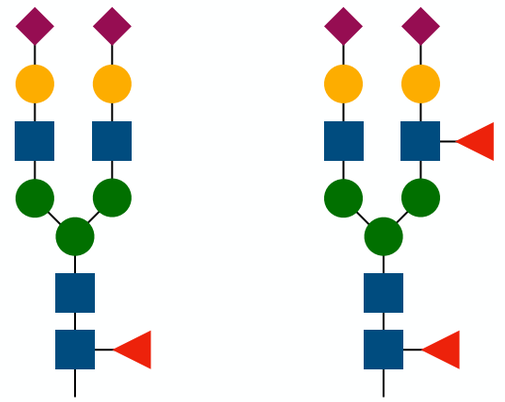
taken from DOI: 10.1126/science.1129594

N-glycan profiles of human v murine

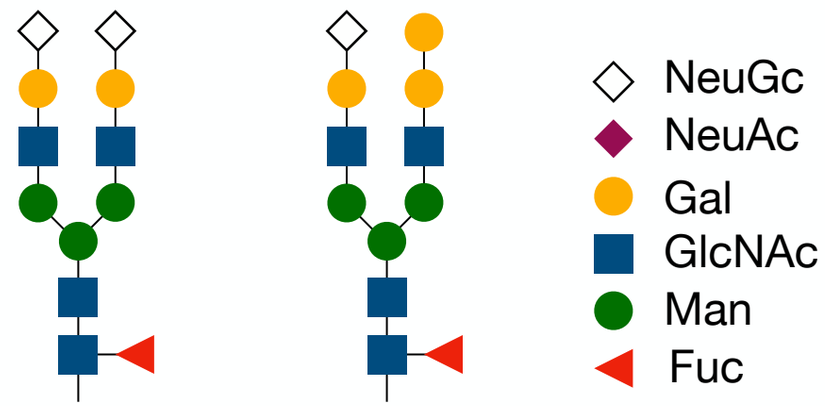
Distinct *N*-glycan between human's and murine's



human neutrophils *N*-glycan



murine neutrophils *N*-glycan

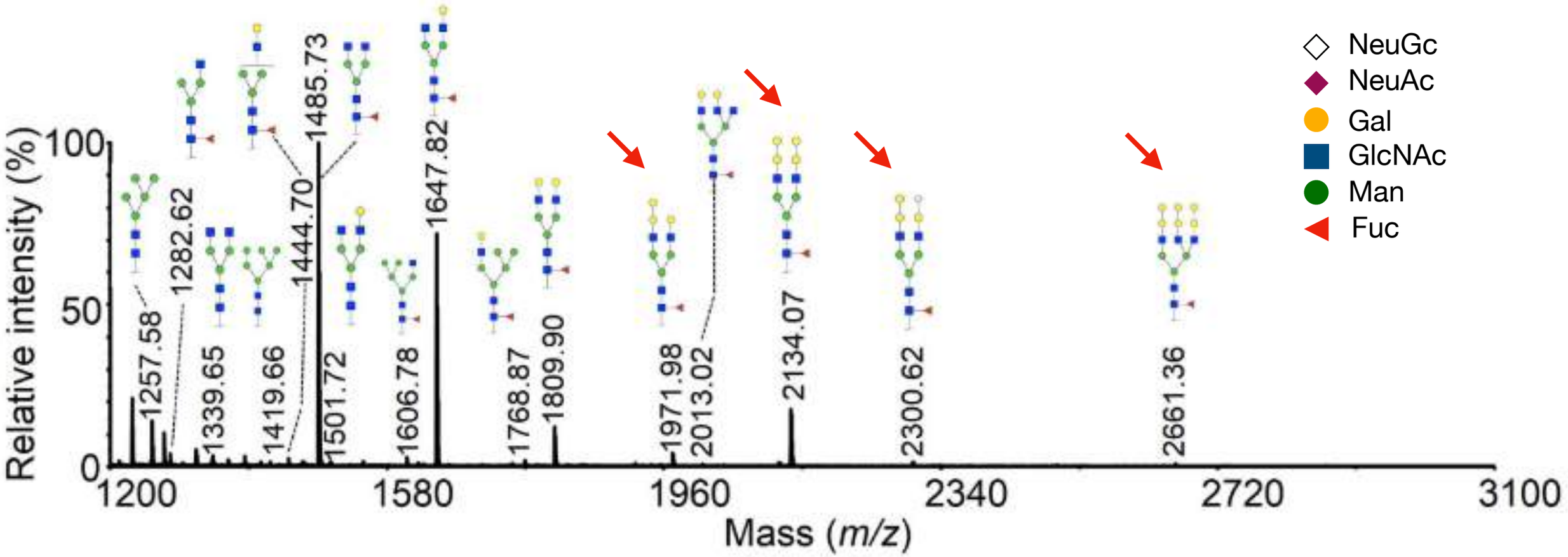


taken from DOI: 10.1042/BST0391334

How about mAb produced in mammalian cell line?

Cetuximab produced in mammalian cell line

non-human type *N*-glycans are exist



Even after the production of chimeric mAb in mammalian cell culture, distinct non-human *N*-glycan motifs are present.

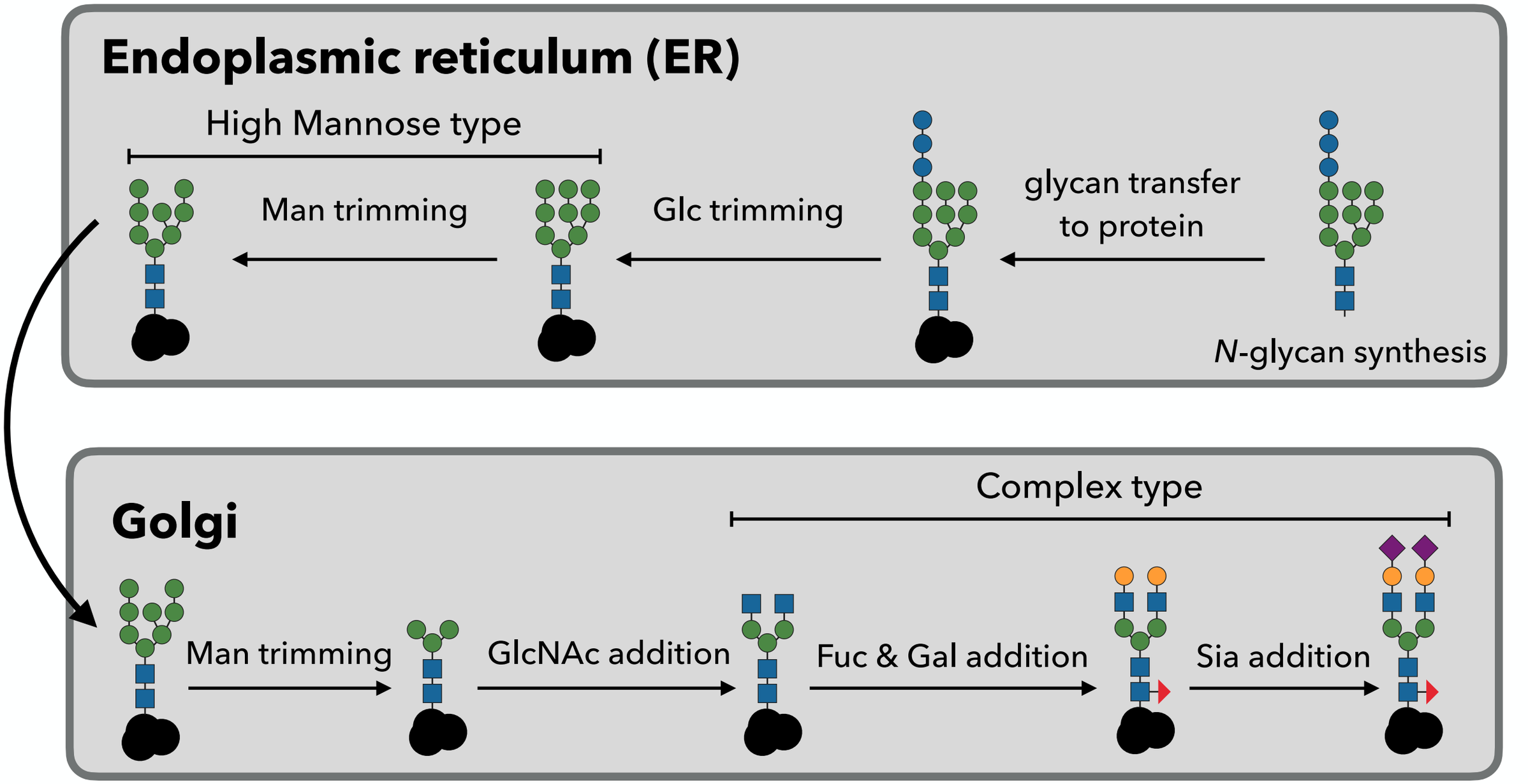
taken from DOI: 10.1371/journal.pone.0170013

Why after all the mAb humanisation effort, still the *N*-glycan is non-human type?

the machine

How *N*-glycosylation works?

N-glycan type depends on its host machinery

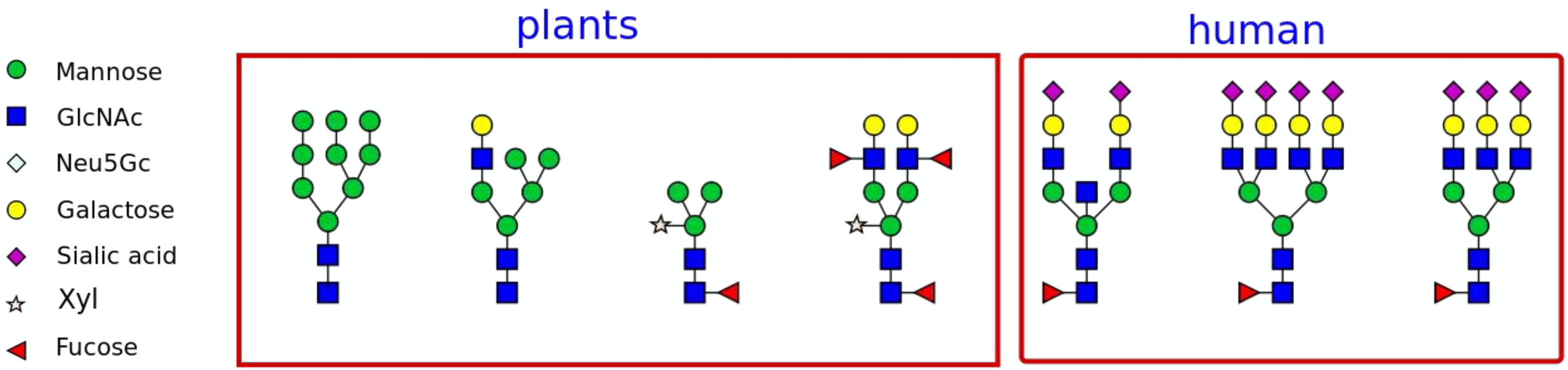


Challenges of plant production system

the *N*-glycan type

Human DNA sequence can be obtained and expressed as a human protein in plant cell.

How about the sugar moieties?



Glyco-engineering to create genetically modified plant expressing human-type *N*-glycan

3. Summary

- Phage display method can be useful for the development of human anti-CoV2 mAb based on V_H & V_L genes of the Covid-19 survivors
- Human protein can be produced in non-human expression platform
- Plant have distinct *N*-glycosylation motifs to that of the human
- Considering the role of *N*-glycan in immunity, therapeutic use of any proteins expressed in plant should be rigorously tested for activity and safety
- Glyco-engineering is necessary to engineer plant *N*-glycosylation machinery to express “more humanised” *N*-glycan

Bonus tracks

What do we do in industrial biotech R&D set-up?

- Recombinant cell line development
- biopharmaceutical analytical method development
- production process development
- bioactivity assay
- scaling up
- biopharmaceutical production method validation
- biologic product stability tests

Thank you!