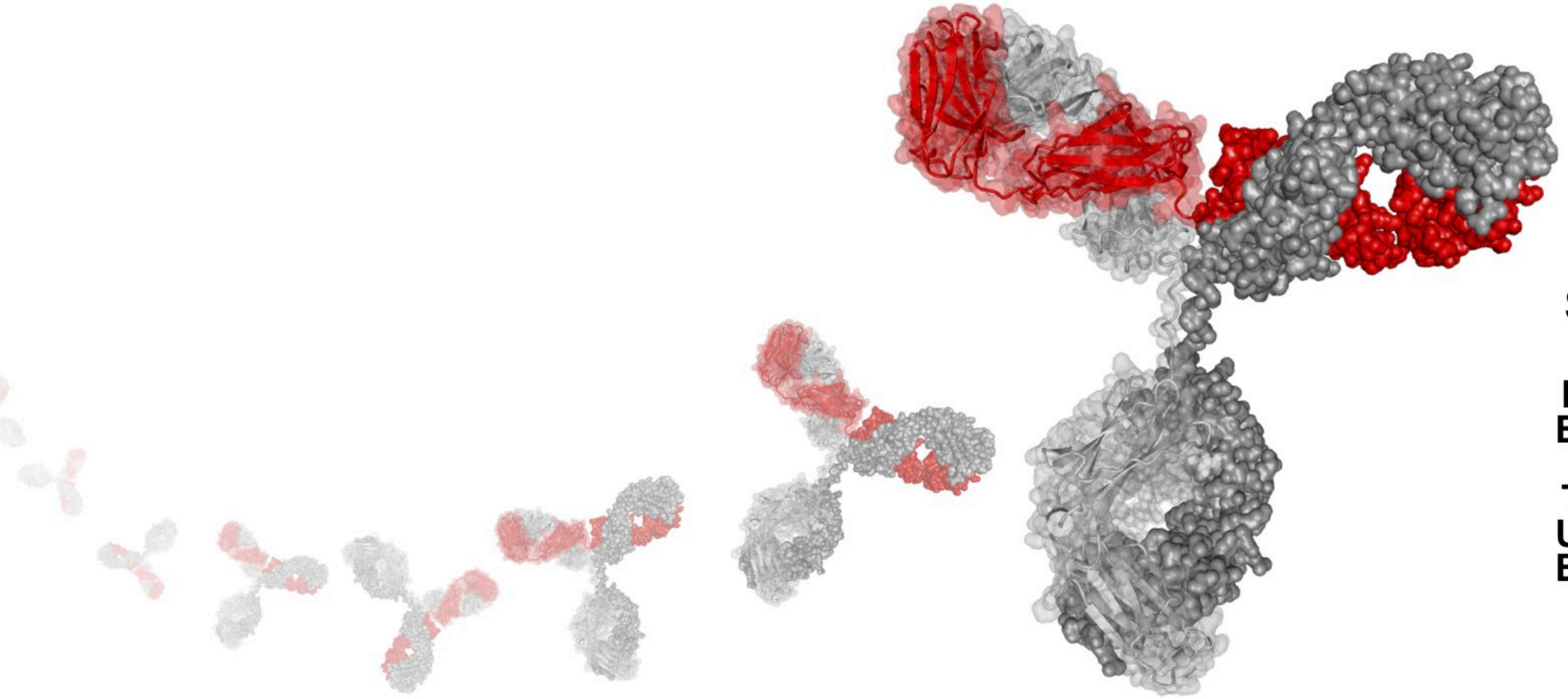
Animal free generation of antibodies



Stefan Dübel

Department of Biotechnology

Technische Universität Braunschweig

Technische Universität Braunschweig

YUMAB Abcalis CORAT

Why NADA (Non-Animal Derived Antibodies)?

NADA offer far more that just animal replacement:

- **Quality**
- **Versatility**
- **Speed**

Quality.

Fact:

NADAs from phage display are most abundantly used in the market segment that requires the highest antibody quality (Therapy)

Unfortunately, they are used to much lower extend for reserach and diagnostics.

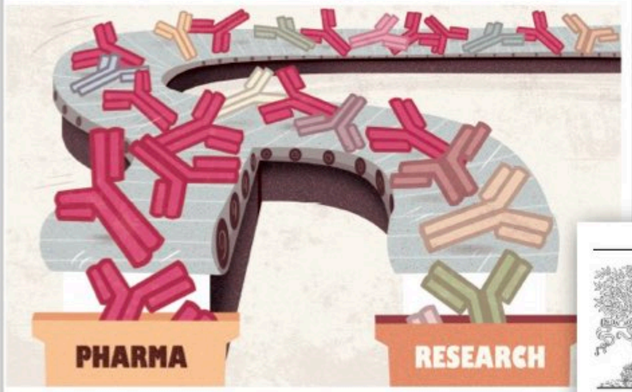
Technische Universität Braunschweig

Shim H. Curr Pharm Des. 2016, 22:6538-59,
& Webseite Antibody Society

Why NADA (Non-Animal Derived Antibodies)?

COMMENT

POLICY Climate engineering research and governance needs to start small p.29 | HISTORY Mysterious defection of cold war physicist revisited p.32 | CORRESPONDENCE Lessons from terrible toll of workaday Typhoon warning p.35 | OBITUARY Mary F Lyon, pioneer of mouse genetics, remembered p.36



Standardize antibodies used in research

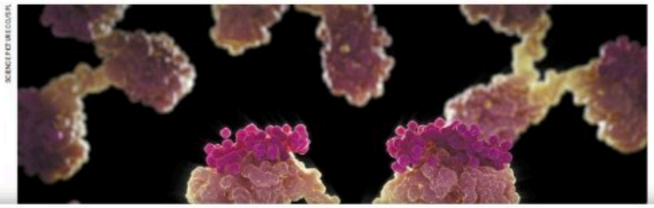
To save millions of dollars and dramatically improve reproducibility, pro… reagents must be defined by their sequences and produced as recombin… pro… say **Andrew Bradbury, Andreas Plückthun** and 110 co-signatories.

Central to reproducibility[1] in biomedical research is being able to use reagents that are identical to those described in publications. Alarmingly, there are serious flaws in the reliability of antibodies, the bind to specific targets. But in a 2008 study[2], fewer than half of around 6,000 routinely used commercial antibodies recognized only their specified targets, with some manufacturers producing consistently good antibodies, and Begley (a co-signatory to this art… able to replicate the scientific res… 6 of 53 landmark preclinical studi… biomedical research, the resultin… materials, time and money is vast…

TECHNOLOGY FEATURE

ANTIBODY ANARCHY: A CALL TO ORDER

Antibodies used in research often give murky results. Broader awareness and advanced technologies promise clarity.



nature

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CORRESPONDENCE · 15 MAY 2020

Reproducibility: bypass animals for antibody production

Alison C. Gray, Andrew Bradbury, Stefan Dübel, Achim Knappik, Andreas Plückthun & Carl A…

…nission's Joint Research Centre has just …endations on non-animal-derived antibodies …2ypgstg), in accordance with the EU's 2010 …ing laboratory animals …d9as). We urge government authorities, …d publishers to endorse this technical …scientific reproducibility and benefit society.

…bodies are plagued by efficacy issues (A. …ckthun *Nature* **518**, 27–29; 2015), with …search reproducibility, diagnosis and health …ntrast, non-animal antibodies derived from …

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The antibody horror show: an introductory guide for the perplexed

Simon. L. Goodman

Translational Biomarkers Research, Translational Innovation Platform – Oncology, Merck KGaA, Frankfurterstr. 250, 64293, Darmstadt, Germany

ARTICLE INFO

Keywords:
Commercial antibodies
Validation
User-Training
Community reporting
Reproducibility

ABSTRACT

The biological literature reverberates with the inadequacies of commercial research-tool antibodies. The scientific community spends some $2 billion per year on such reagents. Excellent accessible scientific platforms exist for reliably making, validating and using antibodies, yet the laboratory end-user reality is somehow depressing – because they often "don't work". This experience is due to a bizarre and variegated spectrum of causes including: inadequately identified antibodies; inappropriate user and supplier validation; poor user training; and overloaded publishers. Colourful as this may appear, the outcomes for the community are uniformly grim, including badly damaged scientific careers, wasted public funding, and contaminated literature. As antibodies are amongst the most important of everyday reagents in cell biology and biochemistry, I have tried here to gently

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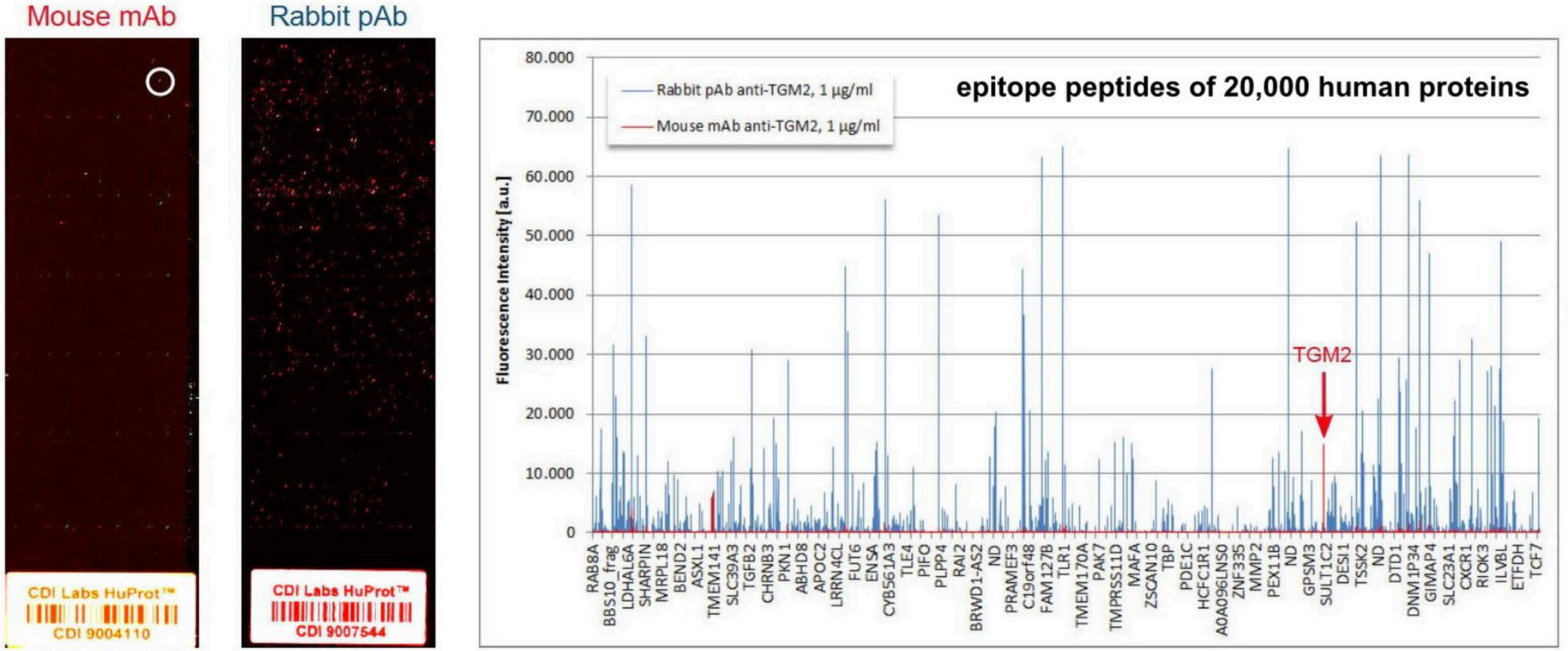
Problems with polyclonals: multiple specificities

The ZEDIRA mouse anti-TGM2 mAb (red / arrow) shows a main response against TGM2. The rabbit anti-TGM2 pAb (Atlas Antibodies, blue) exhibited a strong cross-reactivity on the protein level, but no response against TGM2 protein.

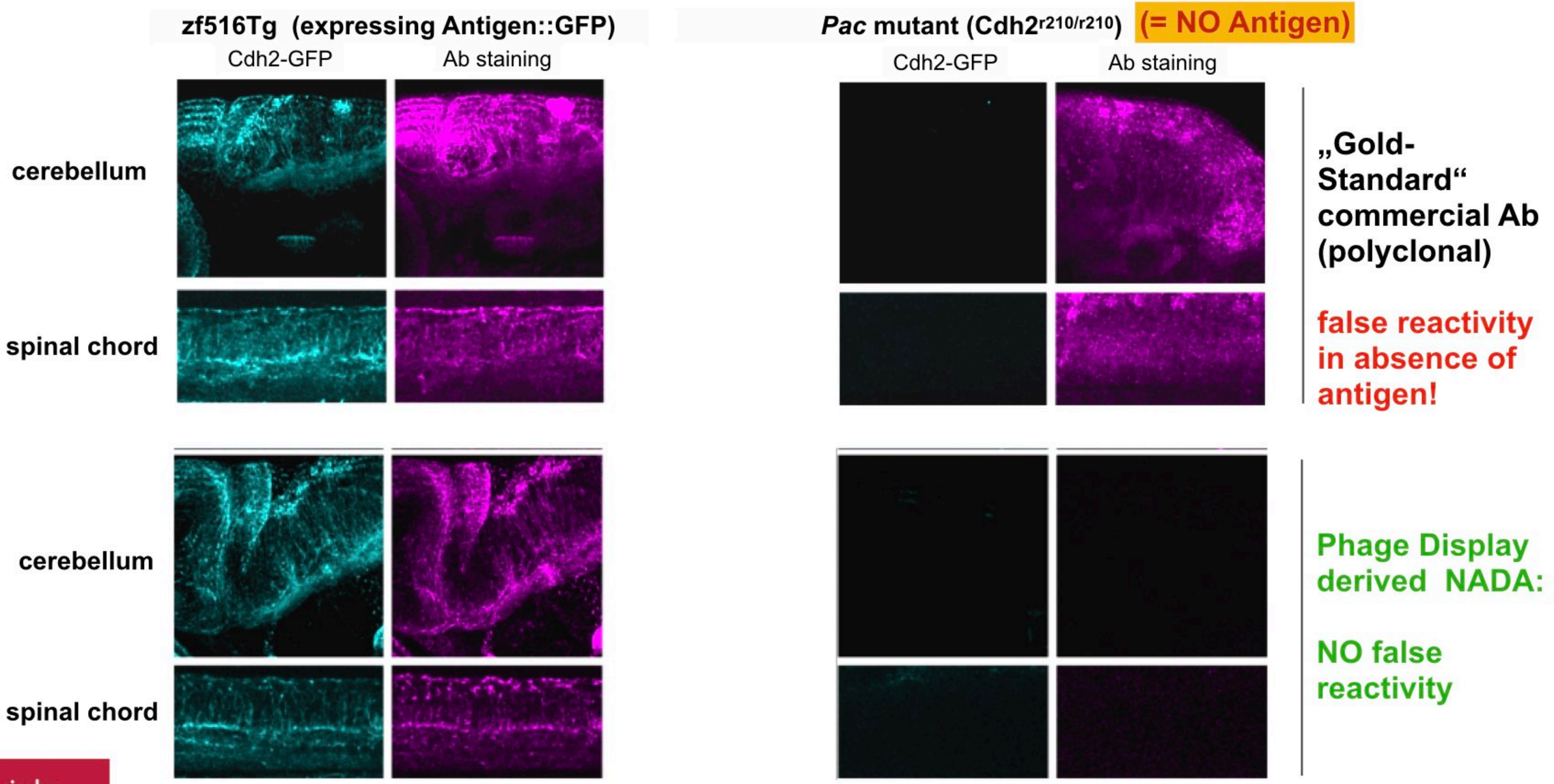


serum
(antibody mixture)

HuProt™ Human Proteome Microarray v3.1 scans and combined intensity plot of the mouse mAb and the rabbit pAb against TGM2.
The mouse anti-TGM2 mAb (red) showed a main response against TGM2 (circled / red arrow) and weaker cross-reactions with the proteins CMIP and JHU07836.P082A01. The highly validated rabbit anti-TGM2 pAb (blue) exhibited a strong cross-reactivity on the protein level, but surprisingly no response against TGM2.

Haber et al., 2019 (poster, PEGS Europe)

Problems with polyclonal reagents, Example: IHC of zebrafish

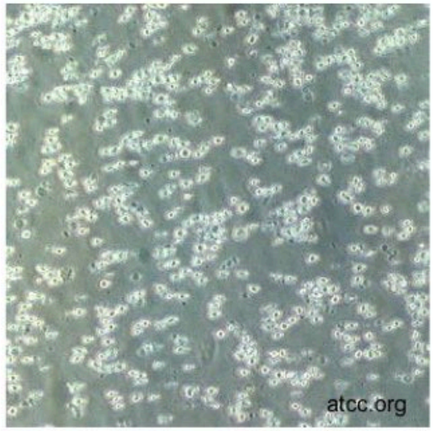


Russo, G. et al., 2018, N Biotechnol.

Monocloals are not always the solution: Productive Antibody-mRNA in Hybridoma mAbs

Hybridoma clones

(moslty of commercial use)



Sequening of 185 hybridomas

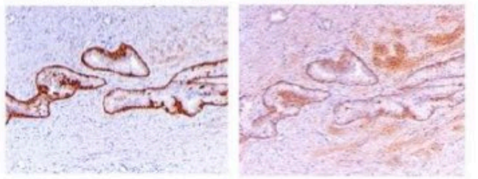
(multicetric study in 7 different labs in 5 countries)

recombinant production
+ Protein A purification

hybridoma supernatants
+ Protein A purification

Specificity testing

Target name	Active Motif prod no.	Purified IgG	EST CONC (ng/mL)	EED	EZH2	Hh3	Hh3.3	P300	POL2 p0	POL2 p52	POL2 p55
EED	61203.0	Hybridoma1	1000 C	186.0	256.8	0.7	0.7	0.8	0.8	0.8	0.8
		Hybridoma1	100 C	9.0	31.3	0.6	0.6	0.7	0.5	0.7	0.6
		VH1 VL1	20 C	104.9	136.6	1.4	1.4	0.9	0.5	0.7	0.8
EZH2	39875.0	Hybridoma2	1000 C	0.2	901.6	4.1	1.2	1.0	0.5	0.5	0.5
		Hybridoma2	100 C	0.4	95.4	0.7	0.7	0.9	0.8	0.7	0.7
		VH1 VL1	20 C	0.3	417.4	0.3	4.3	0.5	0.5	0.5	0.5
		VH2 VL1	20 C	0.5	1.7	1.6	2.1	0.5	0.5	0.5	0.5
H3.3	39677.0	Hybridoma3	1000 C	0.7	827.0	1.8	6.6	1.8	1.3	1.5	1.7
		Hybridoma3	100 C	0.6	42.7	1.0	1.0	1.6	1.0	1.2	1.3
		VH1 VL1	20 C	0.4	67.2	1.2	1.3	0.7	0.6	0.6	0.6
		VH2 VL2	20 C	0.3	8.6	1.6	2.0	77.4	3.3	100.1	774.6
		VH1 VL2	20 C	0.4	1.7	1.3	1.9	0.8	0.8	1.5	2.1
		VH1 VL3	20 C	0.4	0.7	1.5	1.3	0.7	0.7	0.6	0.7
		VH2 VL3	20 C	0.3	0.7	0.9	1.0	0.5	0.5	0.6	0.6
		VH2 VL1	20 C	0.3	0.6	1.1	0.6	0.5	0.7	0.5	1.1
H3total	61475.0	Hybridoma4	900 C	0.6	18.6	60.0	53.5	1.4	1.3	1.3	1.4
		Hybridoma4	90 C	0.6	15.9	2.0	1.8	1.8	1.2	1.3	1.4
		VH1 VL1	20 C	0.6	2.9	58.8	42.2	1.7	1.4	1.3	1.3
		VH1 VL2	20 C	0.5	1.3	2.1	1.7	0.8	1.1	1.0	1.0
pol2	39097.0	Hybridoma5	200 C	2.3	25.1	0.9	1.0	3.3	1.1	15.8	44.9
		Hybridoma5	20 C	0.5	18.5	0.8	0.8	2.1	0.8	7.1	1.4
		VH1 VL1	20 C	0.6	8.5	2.3	3.3	95.1	9.8	71.9	774.6
		VH2 VL1	20 C	0.3	0.9	1.3	1.6	1.4	0.6	0.7	1.7
pol2ser2	61083.0	Hybridoma7	1000 C	0.4	17.9	0.7	0.7	1.0	0.5	29.6	0.8
		Hybridoma7	100 C	0.4	15.1	0.6	0.7	1.3	0.7	0.9	0.8
		VH1 VL1	20 C	0.2	1.3	1.1	1.2	0.7	0.7	434.9	1.5
		VH1 VL2	20 C	0.3	0.7	0.9	1.1	0.6	0.4	0.5	0.6
		VH1 VL3	20 C	0.2	1.3	1.0	1.2	0.4	0.4	0.5	0.5
pol2ser5	61085.0	Hybridoma8	1000 C	0.4	15.8	0.8	0.9	1.0	1.4	21.8	11.7
		Hybridoma8	100 C	0.3	17.2	0.8	0.8	1.2	0.9	7.2	1.0
		VH1 VL1	20 C	0.2	0.7	1.1	1.2	1.5	286.1	783.2	
		VH1 VL2	20 C	0.3	1.2	1.5	1.5	0.8	0.6	1.8	9073.5



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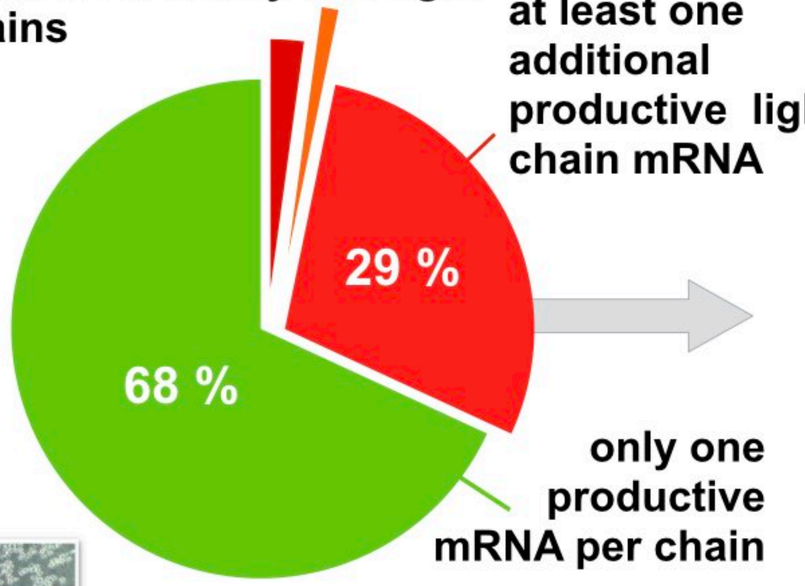
Bradbury et al. (2018) mAbs, *10,* 539-546

Many Hybridoma monoclonals are not monospecific

1%: at least one additional productive heavy chain mRNA

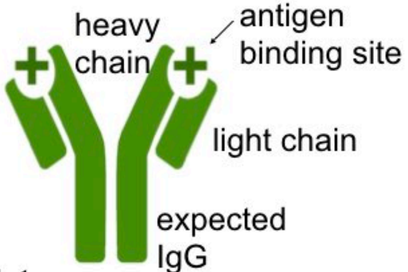
2 %: additional productive mRNAs for heavy and light chains

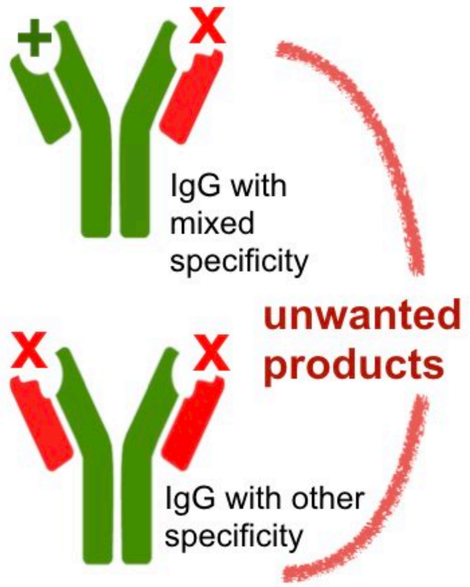


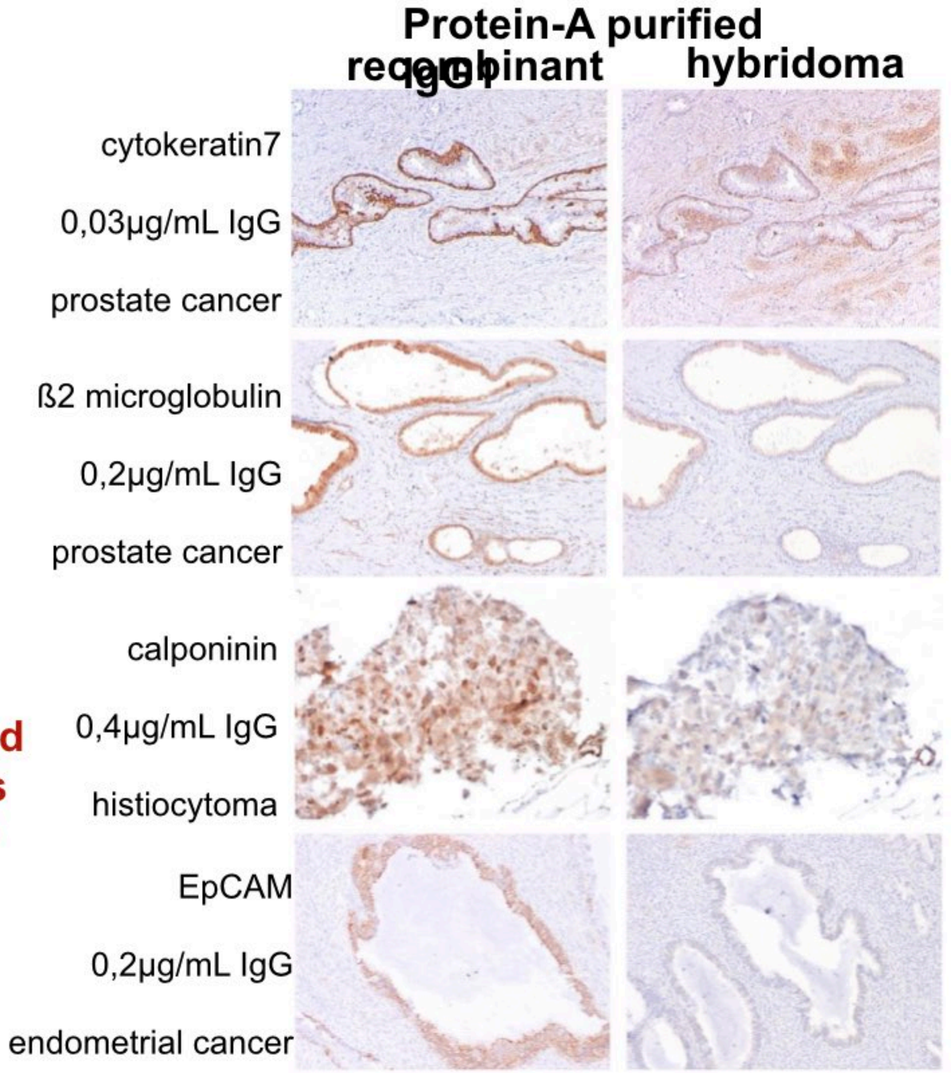
at least one additional productive light chain mRNA

only one productive mRNA per chain

185 hybridomas sequenced
multicetric study (7 different labs in 5 countries)







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Bradbury et al. (2018) mAbs, *10,* 539-546

How to make animal free antibodies:

Most widely established method: phage display

Breitling / Dübel patents (1990/91)

Antibody libraries US Patent 5840 479, EP 0440 146, US Patent 6319 690

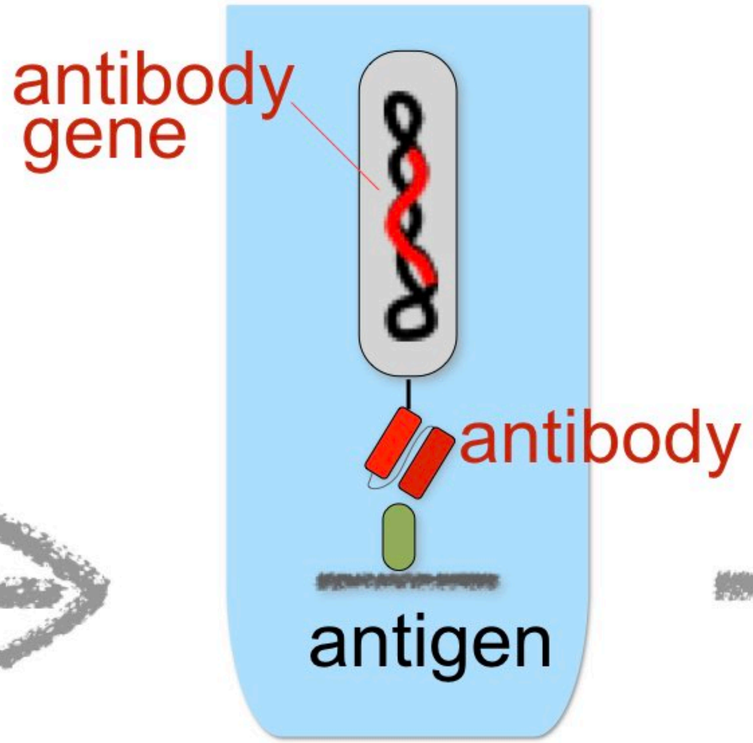
scFv antibody phage display US Patents 5985 588, 5849 500, 6127 132, EP 0547 201

NADA: Non-Animal Derived Antibodies from phage display

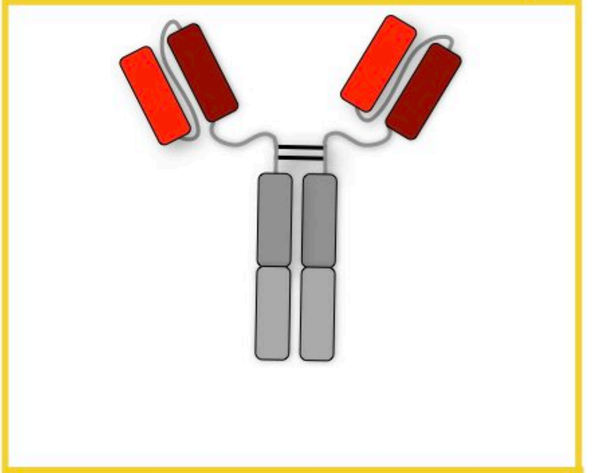
the world's antibody gene repertoire

10,000,000,000 human antibody genes ~

phage panning



completely human antibody



….to any target without immunisation

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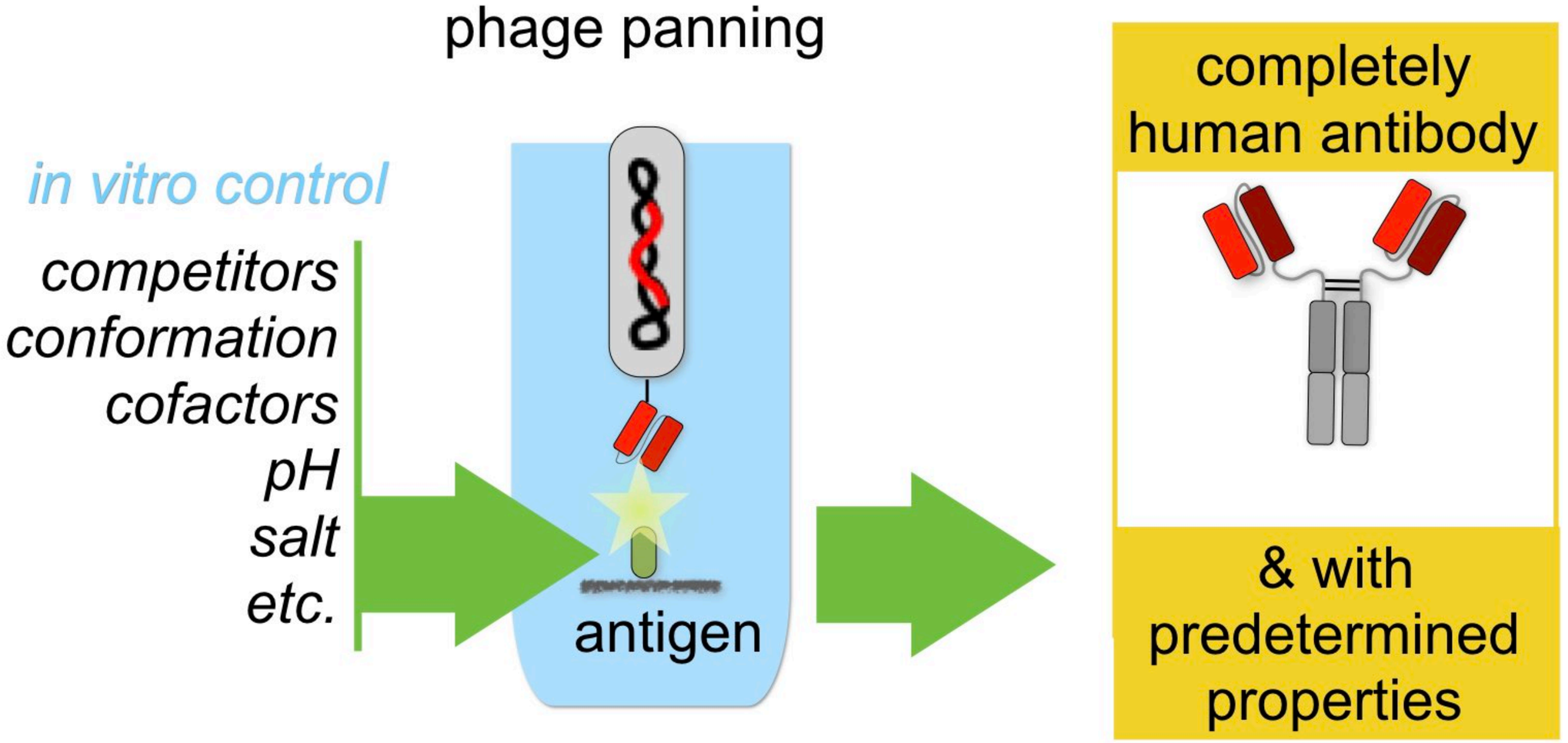
Breitling / Dübel patents (1990/91)

Antibody libraries US Patent 5840 479, EP 0440 146, US Patent 6319 690

scFv antibody phage display US Patents 5985 588, 5849 500, 6127 132, EP 0547 201

NADA: Non-Animal Derived Antibodies from phage display



Breitling / Dübel patents (1990/91)

Animal free antibodies from phage display

EXAMPLES

Breitling / Dübel patents (1990/91)

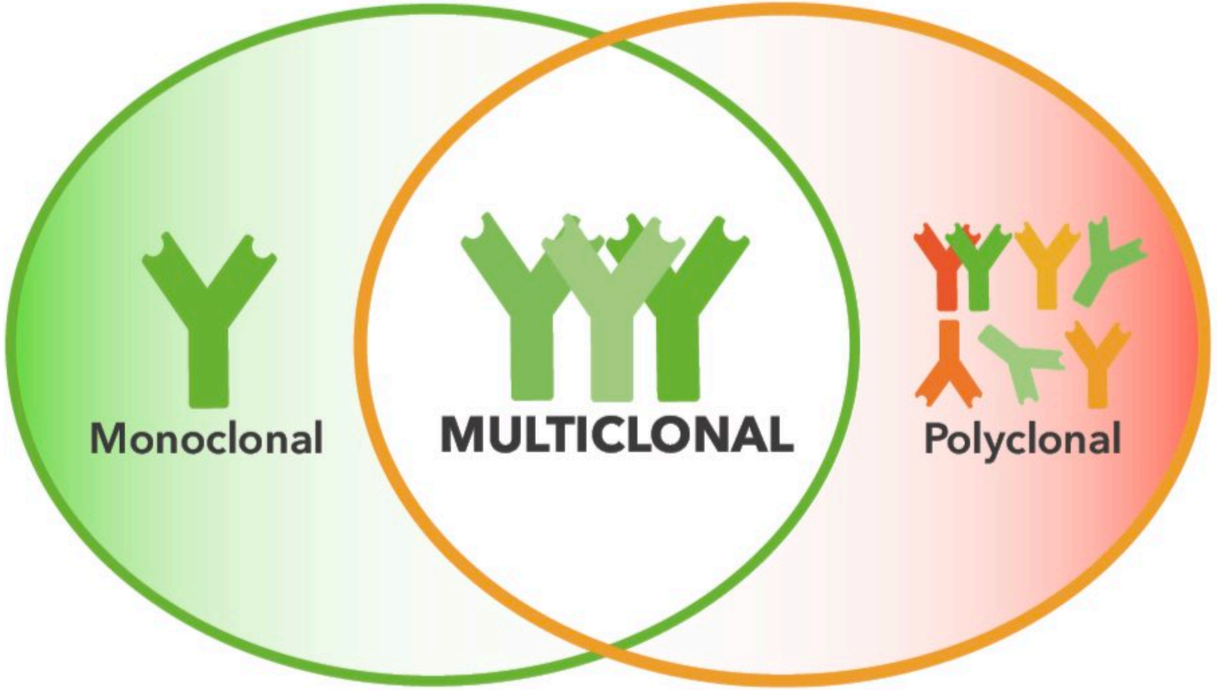
Antibody libraries US Patent 5840 479, EP 0440 146, US Patent 6319 690

scFv antibody phage display US Patents 5985 588, 5849 500, 6127 132, EP 0547 201

Abcalis

MULTICLONAL antibodies:

combining the best of monoclonals and polyclonals



MULTICLONALS:
combining the power of polyclonals and monoclonals

Monoclonal

Polyclonal

- animal use
- recognises one epitope (lower versatility)
- 1/3 of hybridomas contain additional specificities

DISADVANTAGES

ADVANTAGES

ADVANTAGES

DISADVANTAGES

- animal use
- undefined blood product
- limited supply
- unwanted/ unknown reactivities

Determine optimal combination of sequence defined antibodies

ADD ON FEATURES

- No animal use
- composition completely known
- defined epitopes
- no unnown reactivities
- unlimited reagent

- free choice of Fc
- mixture can be adapted to assay

MULTICLONAL

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Gefördert durch:

eXIST Existenzgründungen aus der Wissenschaft

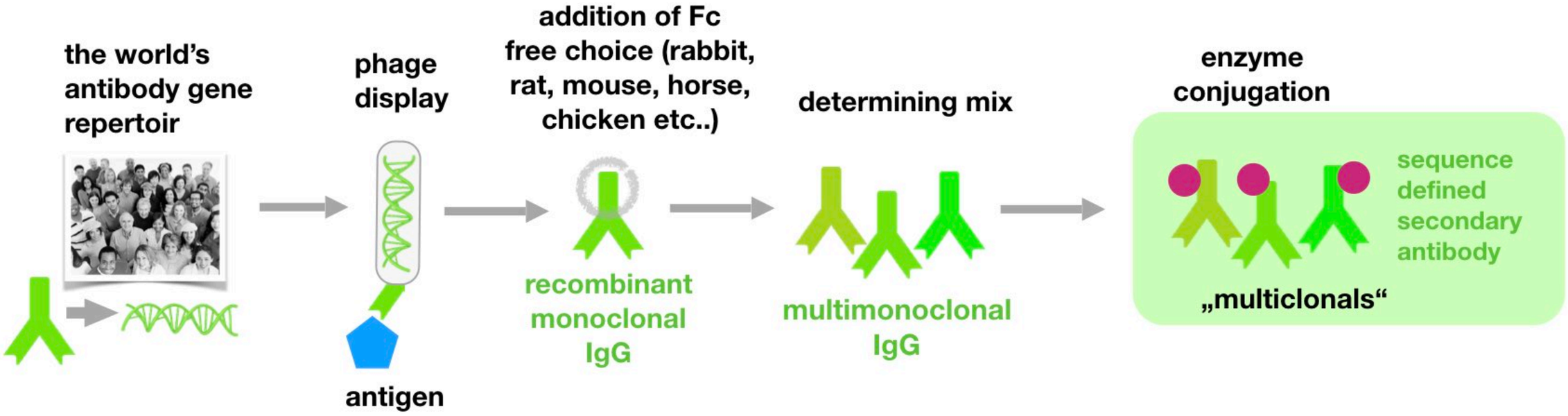
ESF Europäischer Sozialfonds für Deutschland

Europäische Union

Bundesministerium für Wirtschaft und Technologie

aufgrund eines Beschlusses des Deutschen Bundestages

Replacing animal sera: Multimonoclonal secondary antibodies



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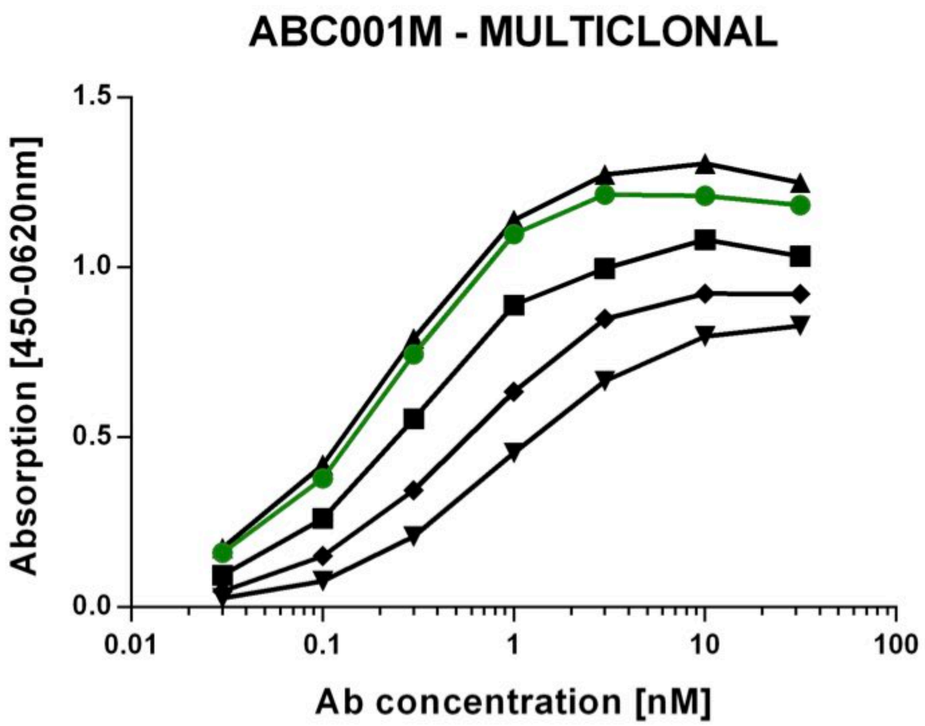
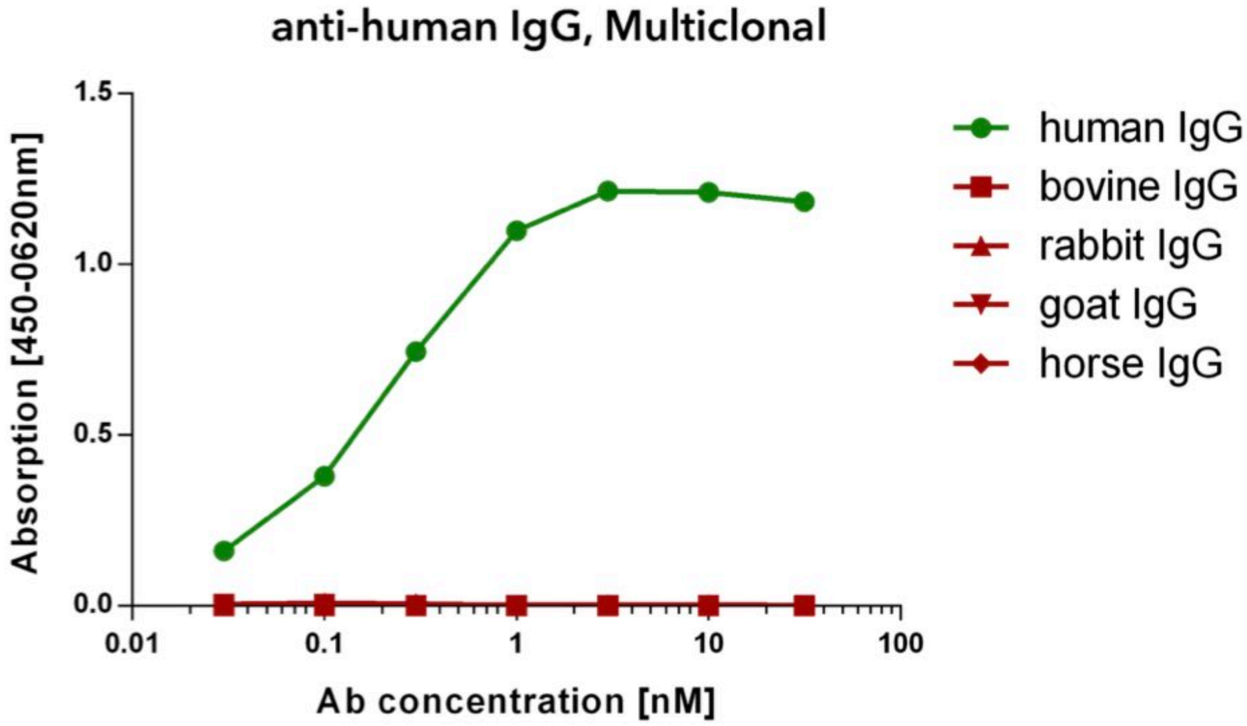
Europäische Union

MULTICLONAL α-hIgG secondary antibody: Specificity





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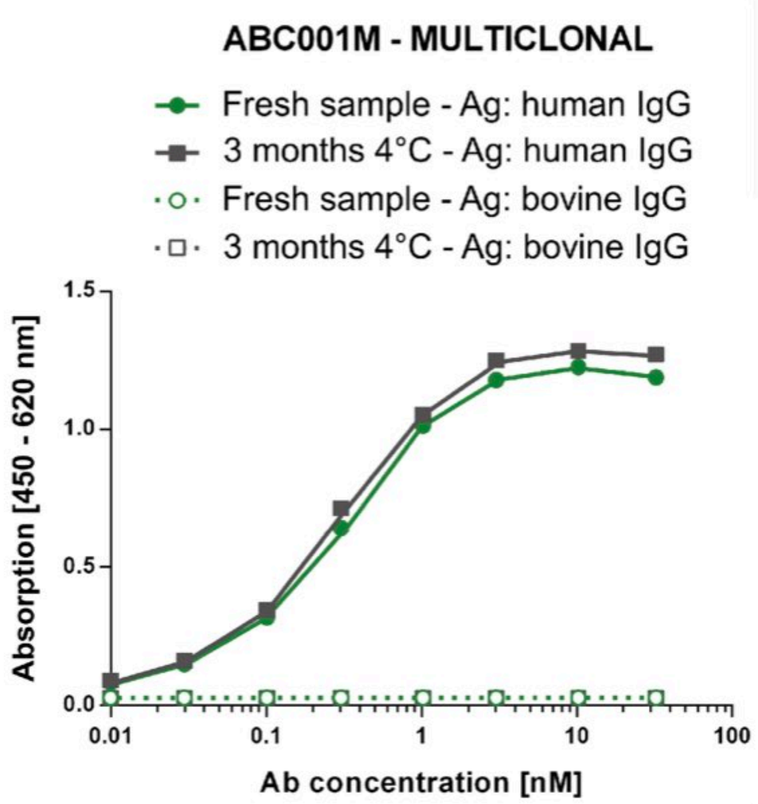
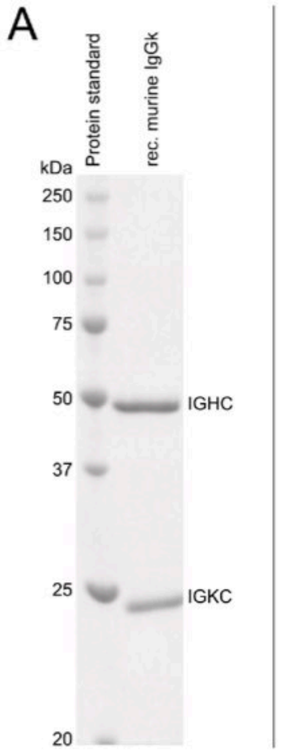
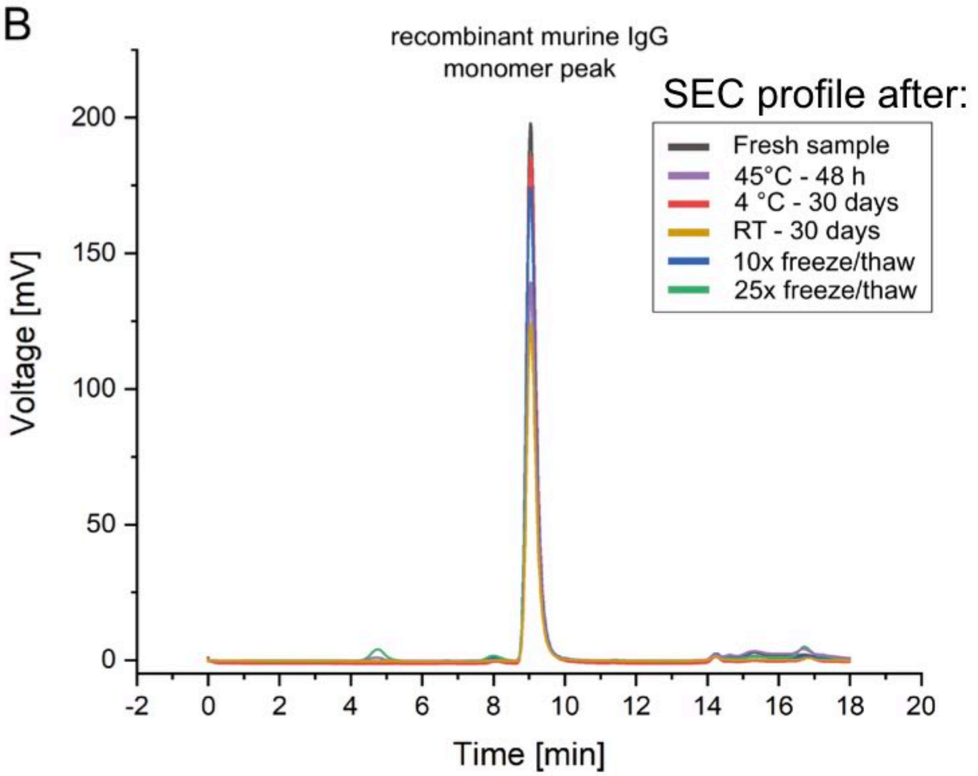
Composing defined multiclonals (Example): stability

storage stability





freeze-thaw (up to 25x) stability



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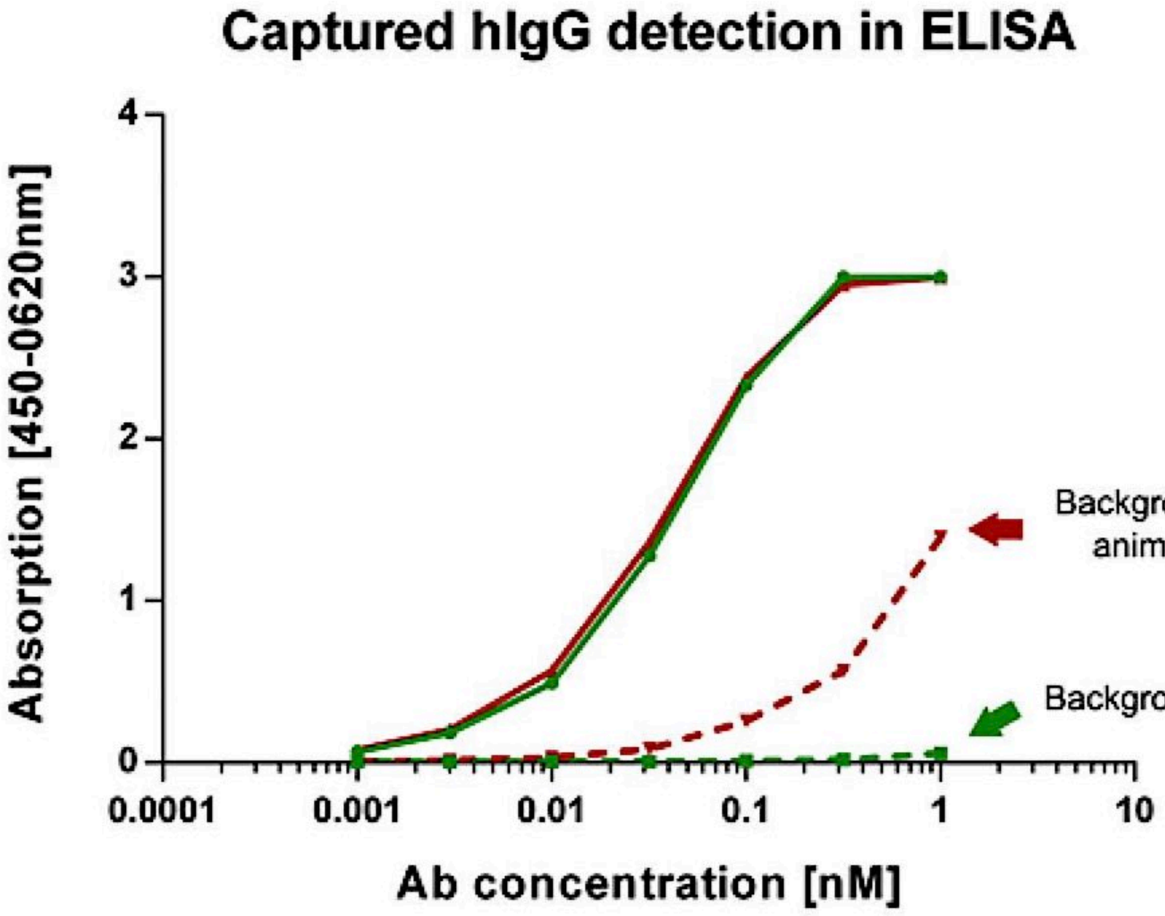
MULTICLONAL α-hIgG: lower Background



anti-hIgG antibody:

- Multiclonal-HRP
- Goat polyclonal-HRP

Background binding of typical polyclonal animal derived secondary antibody

Background binding of Abcalis Multiclonal

ADA serum (catalog product) (= undefined antibody mixture)

NADA ATGCAGTCCTAAATTAGG.. (selected equence defined antibodies)

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MULTICLONALS - Summary

- completely animal free (discovery & production)
- recognize several defined epitopes
- provide „polyclonal“-like signal amplification
- tested to be robust reagents
- lower unwanted reactivity
- can be made with Fc part of choice
- provide unlimited reproducibility
- available today

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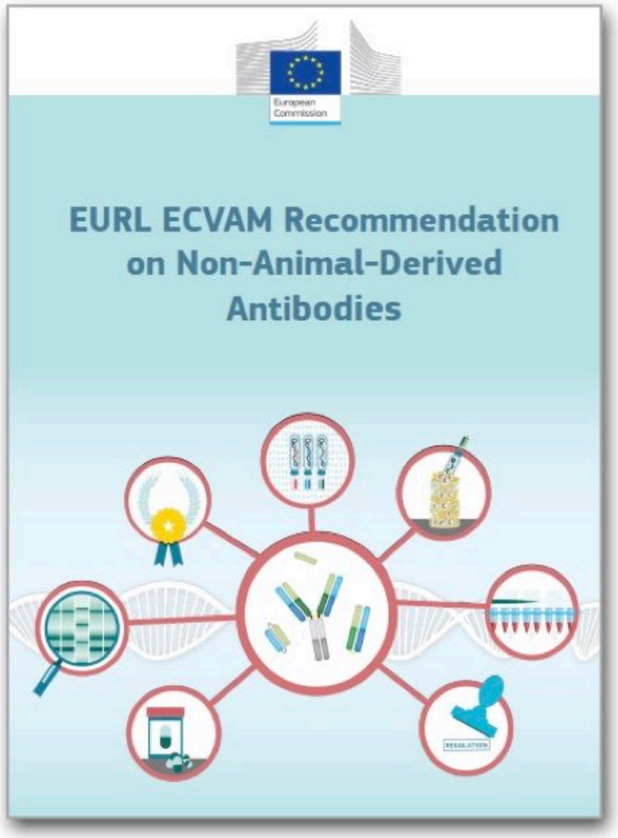
Our Multiclonals are fully compliant with EU guidelines



https://publications.jrc.ec.europa.eu/repository/handle/JRC120199

toxicity tests or for the quality control of biologicals, should be non-animal derived. This will enhance reproducibility of results and the sustainable supply of reagents.

Animal-derived polyclonal antibodies make up a large proportion of animals used today for antibody production and, therefore, present serious ethical concerns. However, they can be produced using defined mixtures of sequence-defined recombinant antibodies developed from universal phage display libraries, thereby avoiding the use of animals. These so-called "multiclonal" antibodies[2] have been recently shown to exceed the performance of the monoclonal products[3]. These recent developments also show that non-animal-derived "multiclonal" antibodies with superior quality (e.g., lower unspecific reactions) and higher reproducibility over animal-derived polyclonal antibodies can and should be generated. It is therefore possible to combine the best features of monoclonal and polyclonal antibodies in a completely animal-free and defined product.

Even though the ESAC review did not cover the field of therapeutic applications, EURL ECVAM considers that non-animal-derived antibodies are also a suitable alternative in this field. In fact, monoclonal affinity reagents approved for therapeutic applications are nowadays exclusively recombinant, well characterised because of strict regulations, and stably produced in large amounts. However, while several of these affinity reagents are

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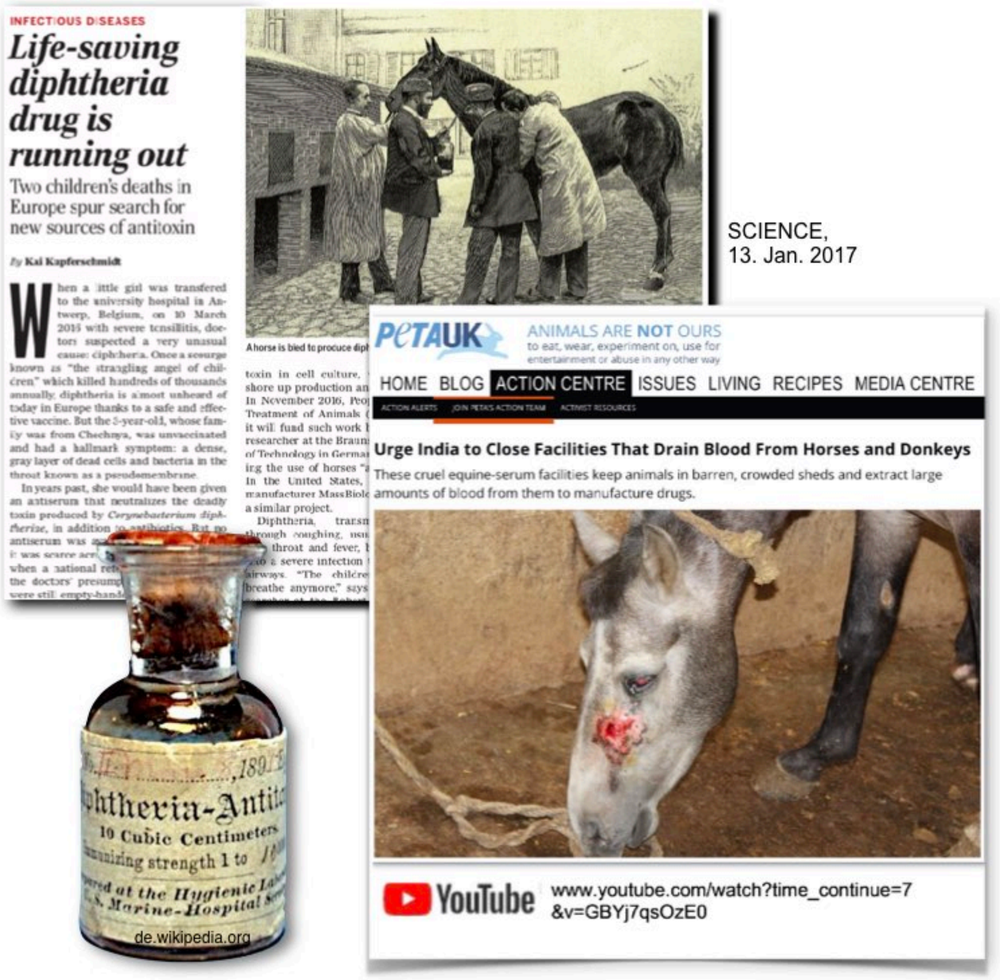
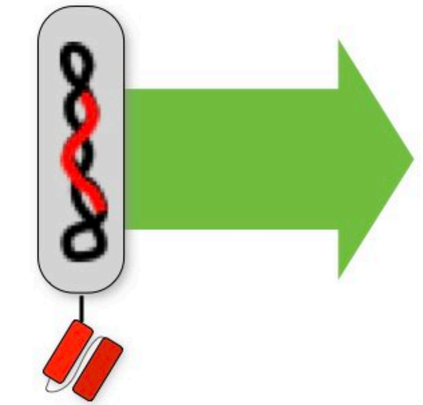
Example 2: NADA to replace polyclonal horse sera against Diphtheria



SCIENCE,
13. Jan. 2017

PETA INTERNATIONAL SCIENCE CONSORTIUM LTD.



phage display

- ☑ >600 human antibodies against Diphtheria toxin
- ☑ best ones: 9x better neutralizing than best horse serum and monoclonal NIBSC world standard

Technische Universität Braunschweig

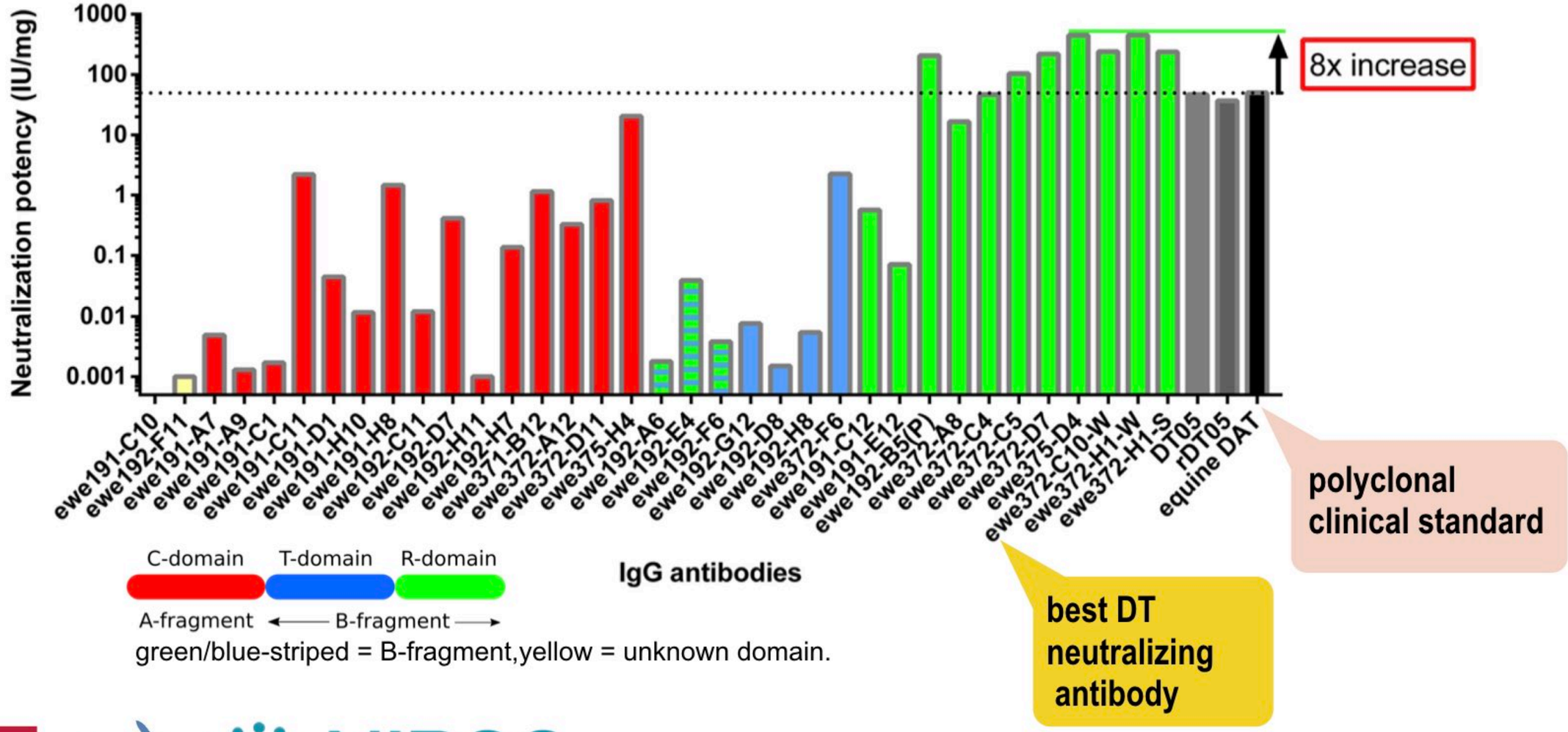
PISC

NIBSC Confidence in Biological Medicines

Wenzel, et al. (2020) Sci. Rep. 10, 571

Animal free antibodies are better neutralising that clinical animal sera

Neutralization potency of IgG antibodies expressed as IU/mg determined by Vero cell neutralization assay with a toxin dose level of 4 Å~ MCD.



green/blue-striped = B-fragment,yellow = unknown domain.

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PISC

NIBSC Confidence in Biological Medicines

Wenzel, et al. (2020) Sci. Rep. 10, 571

Versatility.

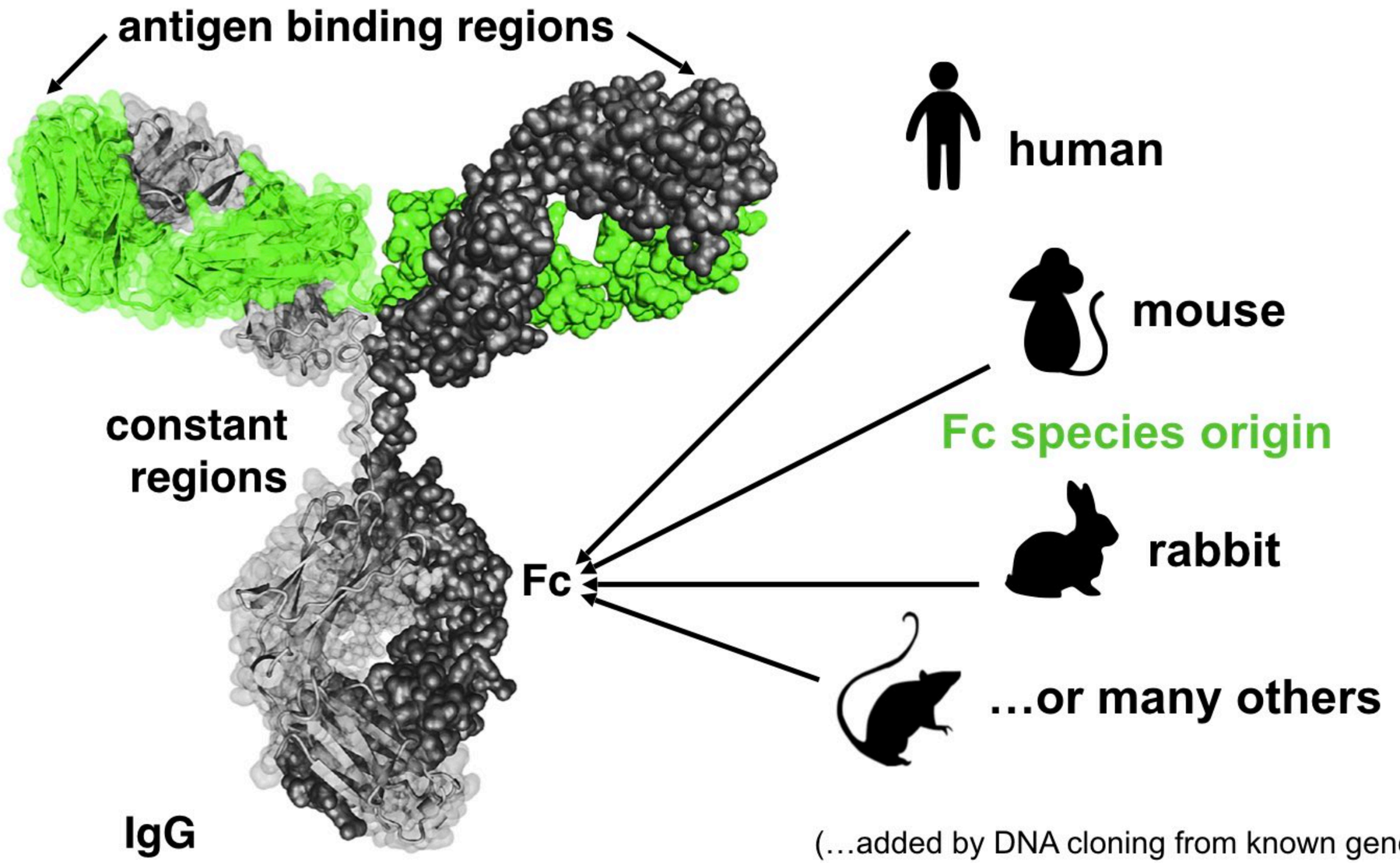
Human (animal-free) antibodies from universal HAL libraries

origin of antigen	number of different antigens	number of unique, validated binders
human	336	2375
other mammalia	43	288
metazoa	21	148
plant	14	51
fungi	13	41
bacteria	70	457
virus	14	61
haptens	6	20
total	**517**	**3441**

1/2020

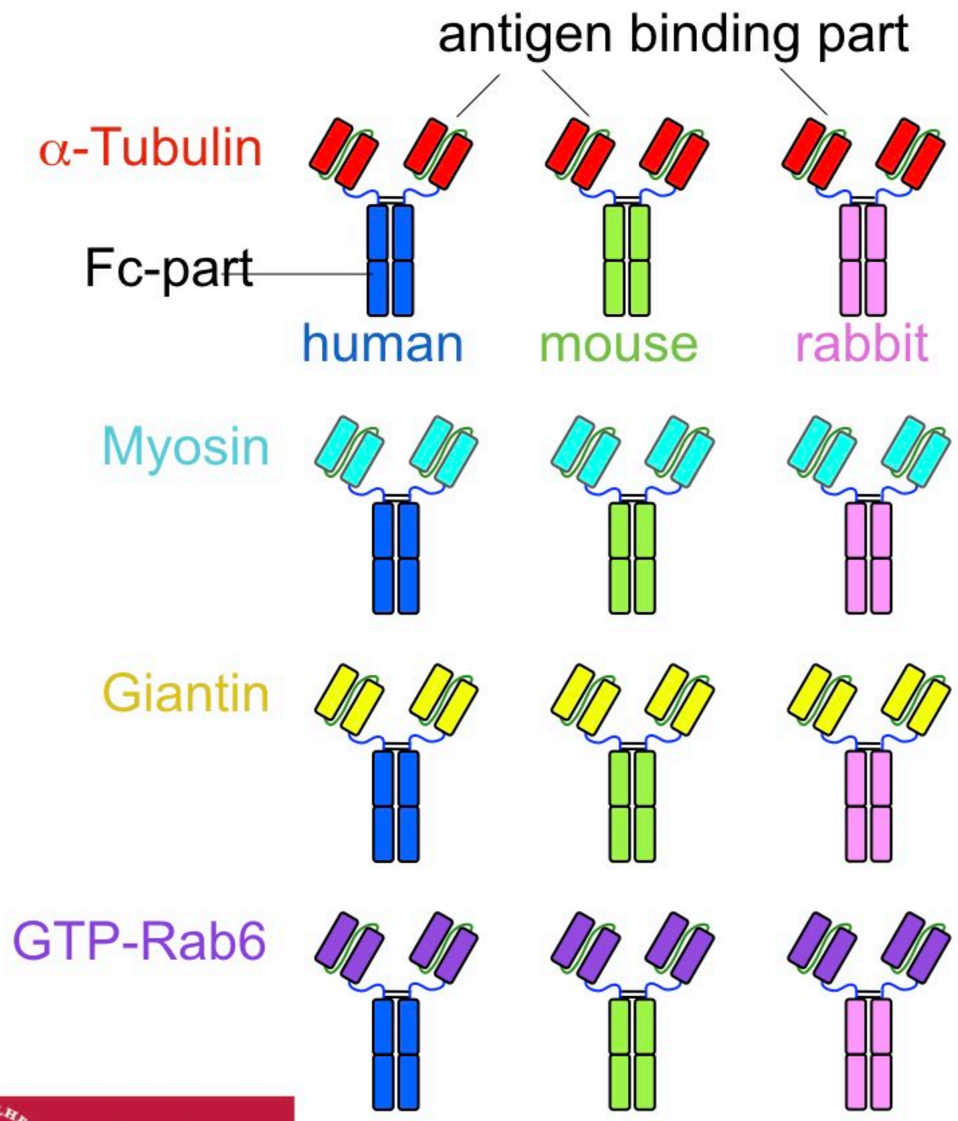
from diversity > $1.5 \times 10^{10,}$ naive, close to germline human antibody phage display libraries

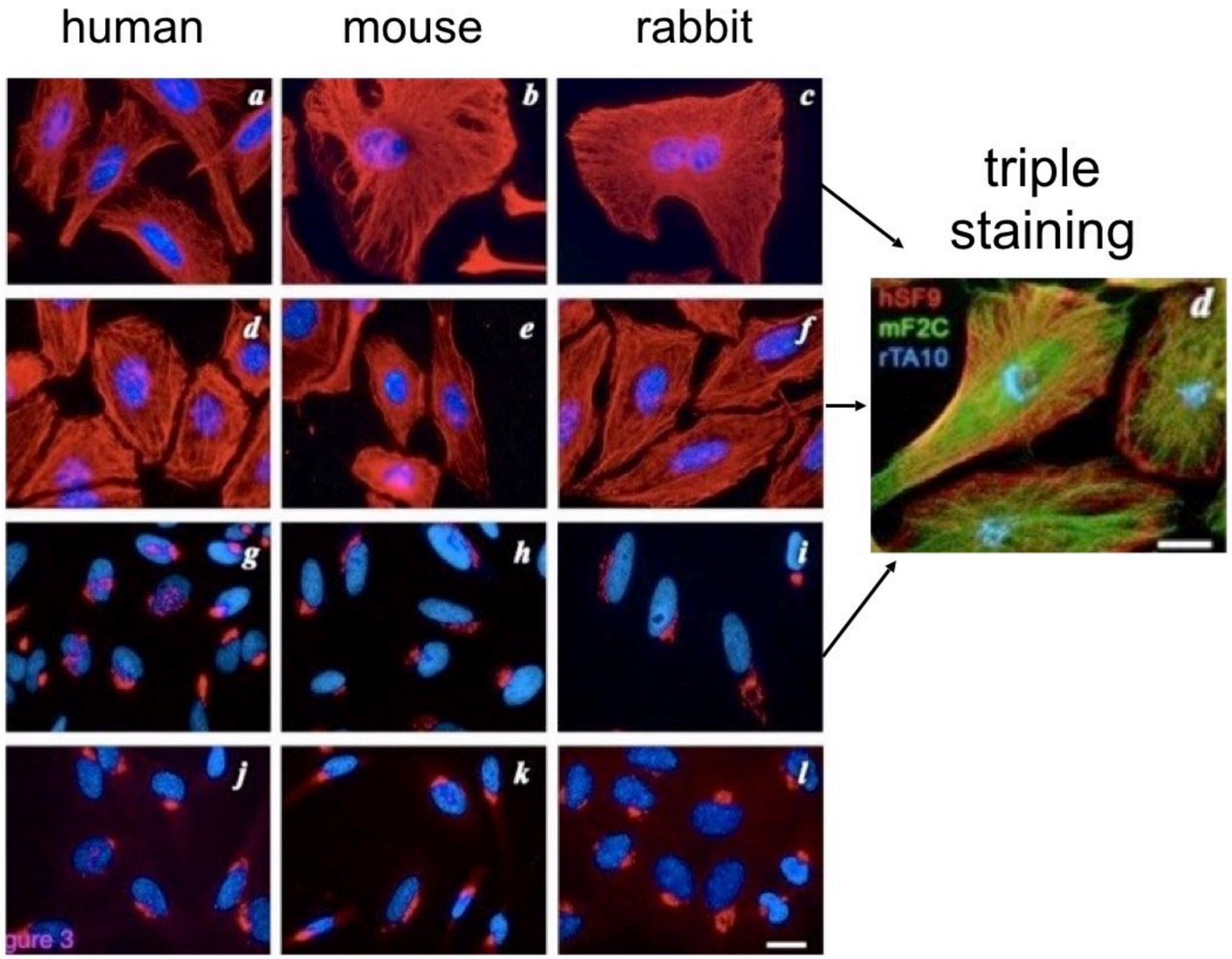
Benefits of recombinant antibodies: Free choice of Fc part



(…added by DNA cloning from known genes)

Engineered antibodies with Fc of differerent species:

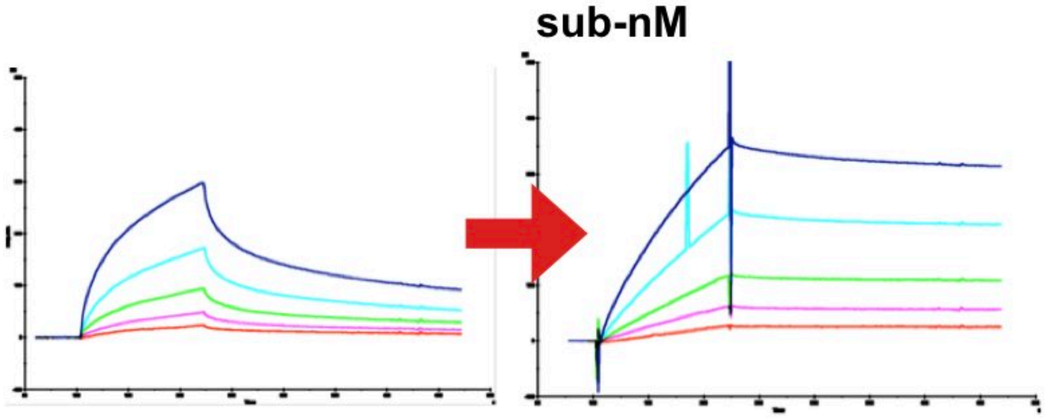




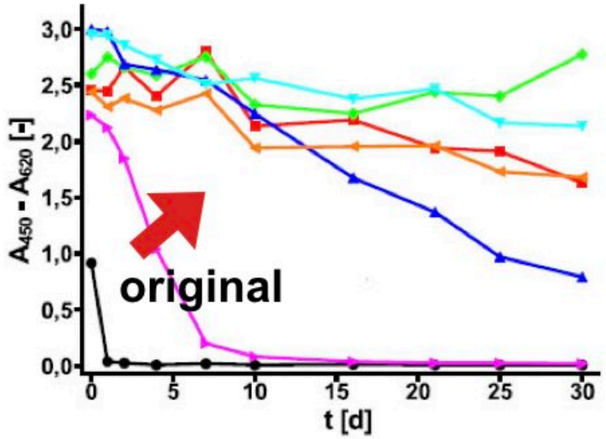
Franck Perez and coworkers, Moutel *et al*., 2009 BMC Biotechnology, 9:14

Improving antibodies by *in vitro* evolution

- **>500 fold affinity maturation**

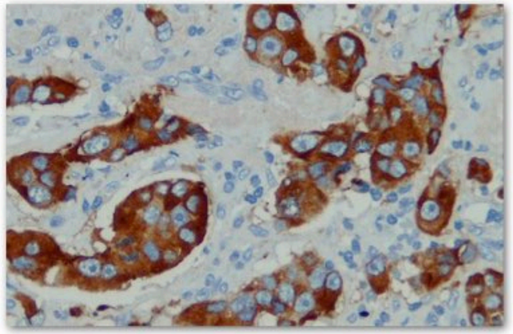


- **increased stability**

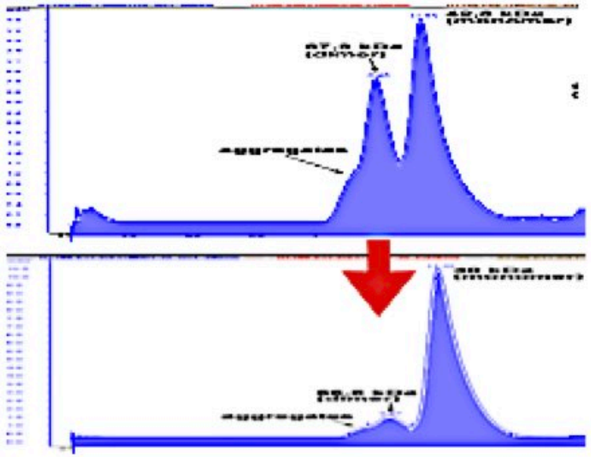


end product:
stable >1 month
37°C serum

Anti-mamma carcinoma antibody



- **less aggregation**



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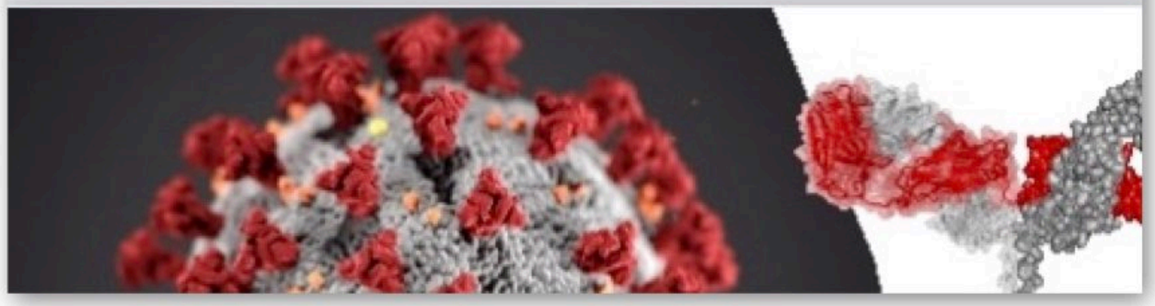
Thie et al. (2011) PLoS ONE vol.6, e15921

Speed.

Rapid animal-free antibody generation to SARSCoV2

Yumab GmbH
820 followers
3 Monate · Bearbeitet

In cooperation with #Boehringeringelheim and in less than 4 weeks, YUMAB generated and characterized the first human antibodies with #receptor_blocking_activity against the new #Coronavirus by applying the Nobel prize awarded #phage_display Technology.
Thanks to all involved for their excellent work.
By providing these antibodies, the first step is taken towards a therapeutic antibody to combat #Covid19.
https://lnkd.in/dmjSC9E
#Coronavirus #SARS_CoV2 #Covid19



nature methods

level[10] (Edwards et al., 2018). The dawn of a new era is upon us where neither the scientific nor the ethical shortcomings of animal-derived antibodies need be tolerated any longer. A poignant and pertinent example of this is the astonishingly rapid generation of animal free, human monoclonal antibodies to SARS-CoV-2 in response to the urgent need for solutions to counteract the spread of the virus by numerous biotech companies worldwide. Whilst some groups are relying on immunisation strategies, others are using non-animal approaches including human B cells from convalescent sources or large pre-existing naive antibody libraries to select antibodies by phage or yeast display, permitting the generation of antibodies in as little as 4 weeks[11,12]

Gray, et al. (2020) The impact of EU policy on antibody generation. *Nature Meth. in press.*

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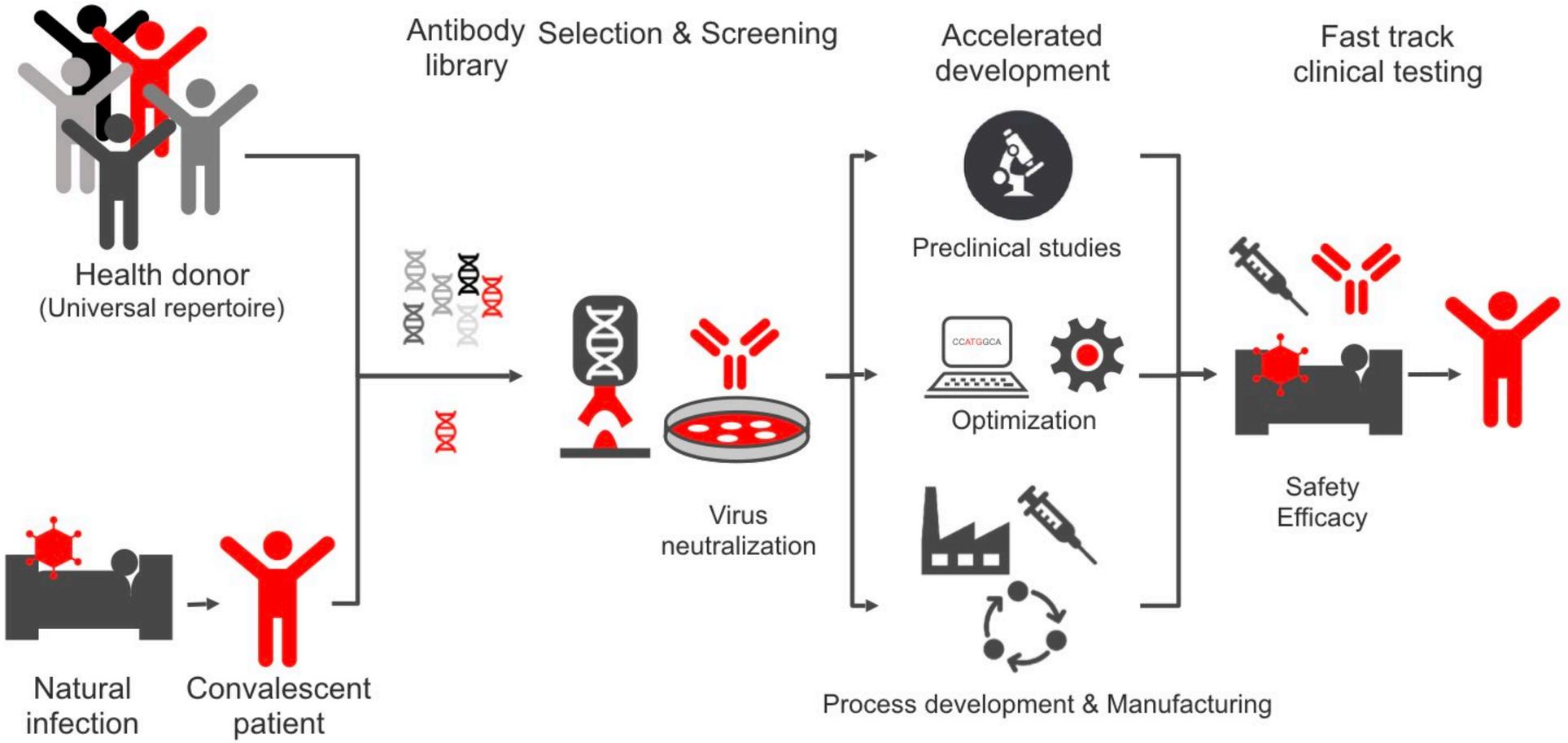
Rapid human antibody generation to SARSCoV2



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corat-therapeutics.com

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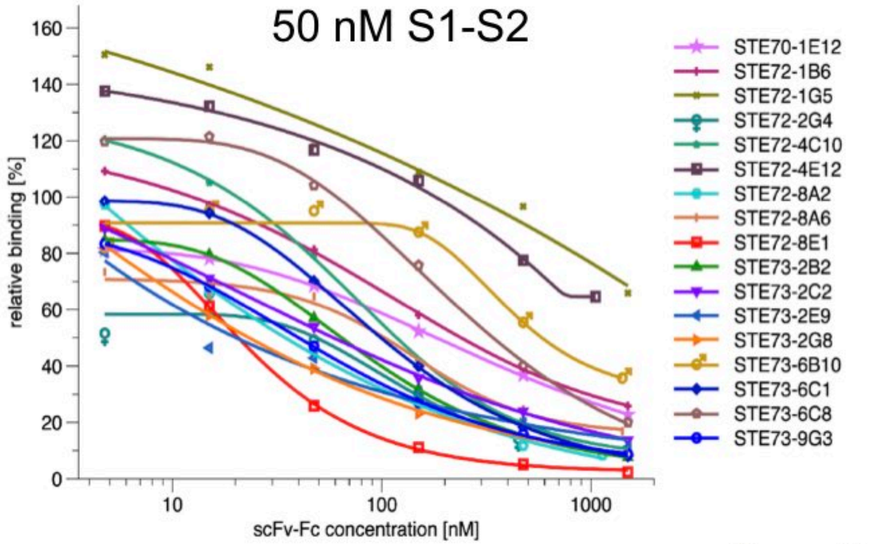
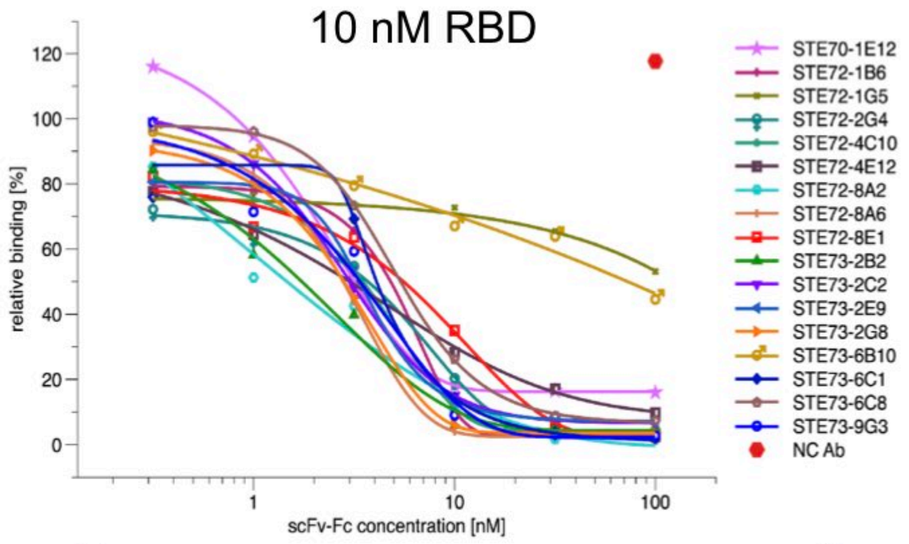
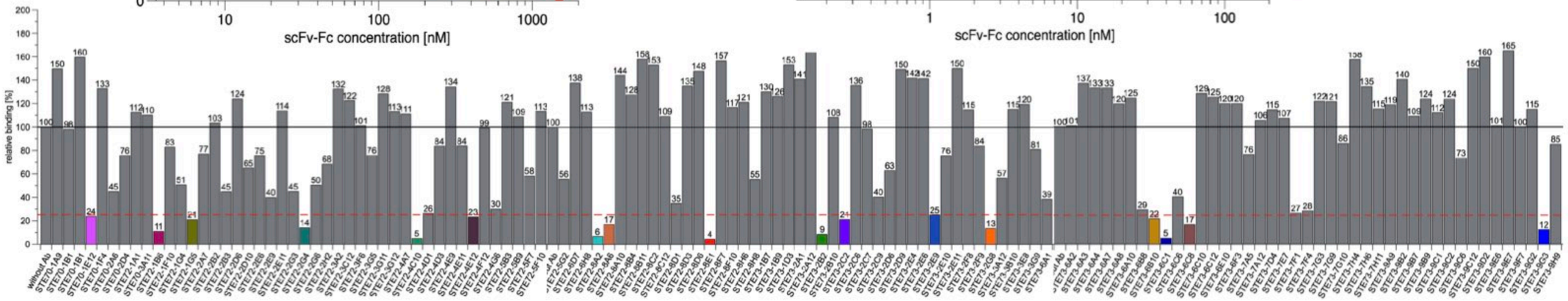
SARS-CoV-2 inhibiting antibodies from nonimmunized libraries

IC50 determination by flow cytometry





virus inhibition screen



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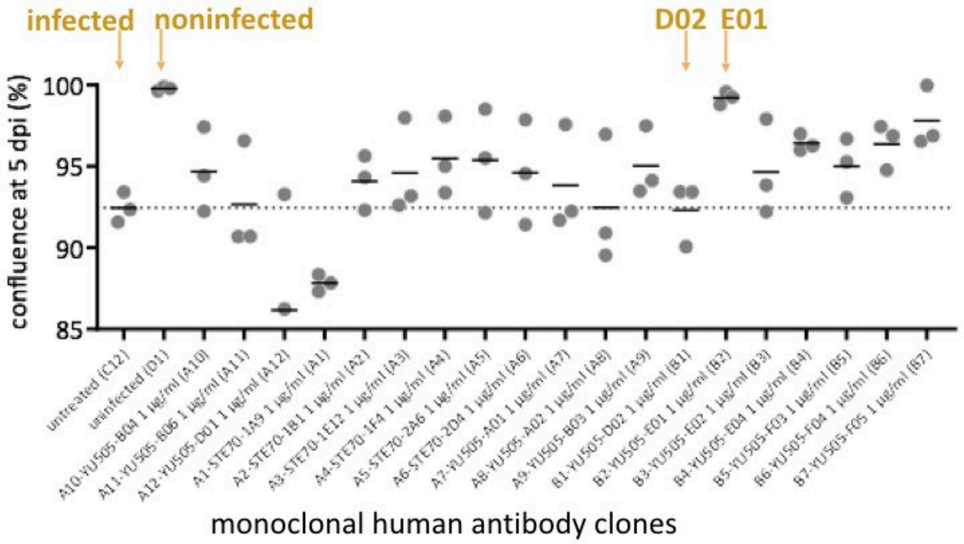
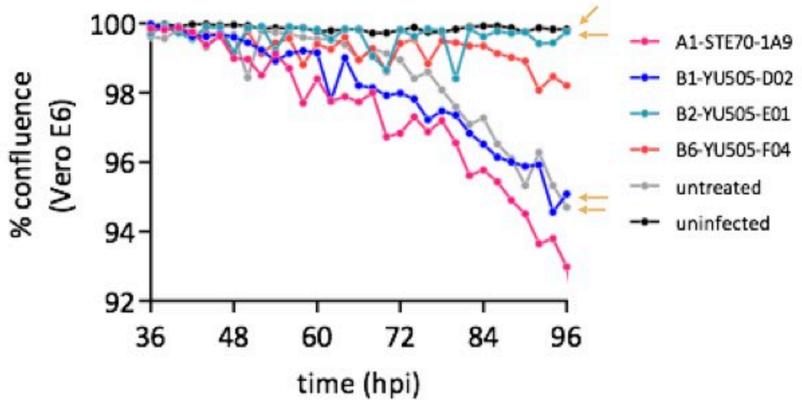
Protective antibodies against SARSCoV2 (animal free)



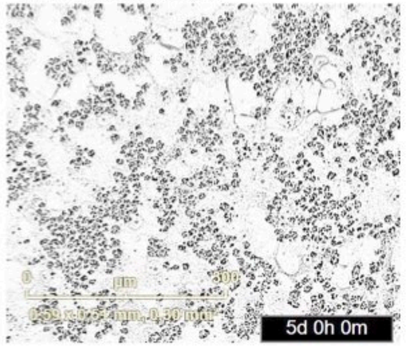
monoclonal human antibody clones



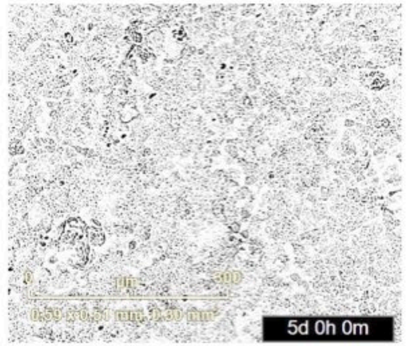
Virus growth kinetics

arrows mark samples corresponding to the microscopy panels

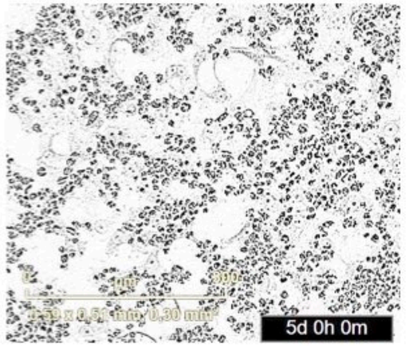
SARS-CoV-2 infected, untreated



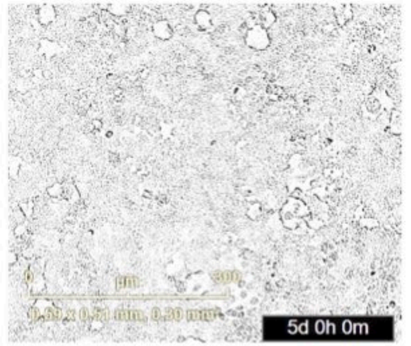
no Virus (healthy cells)



SARS-CoV-2 – +mab YU505-**D02**



SARS-CoV-2 – +mab YU505-**E01**



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HELMHOLTZ ZENTRUM FÜR INFEKTIONSFORSCHUNG

Data: Ulfert Rand, Luka Čičin-Šain HZI

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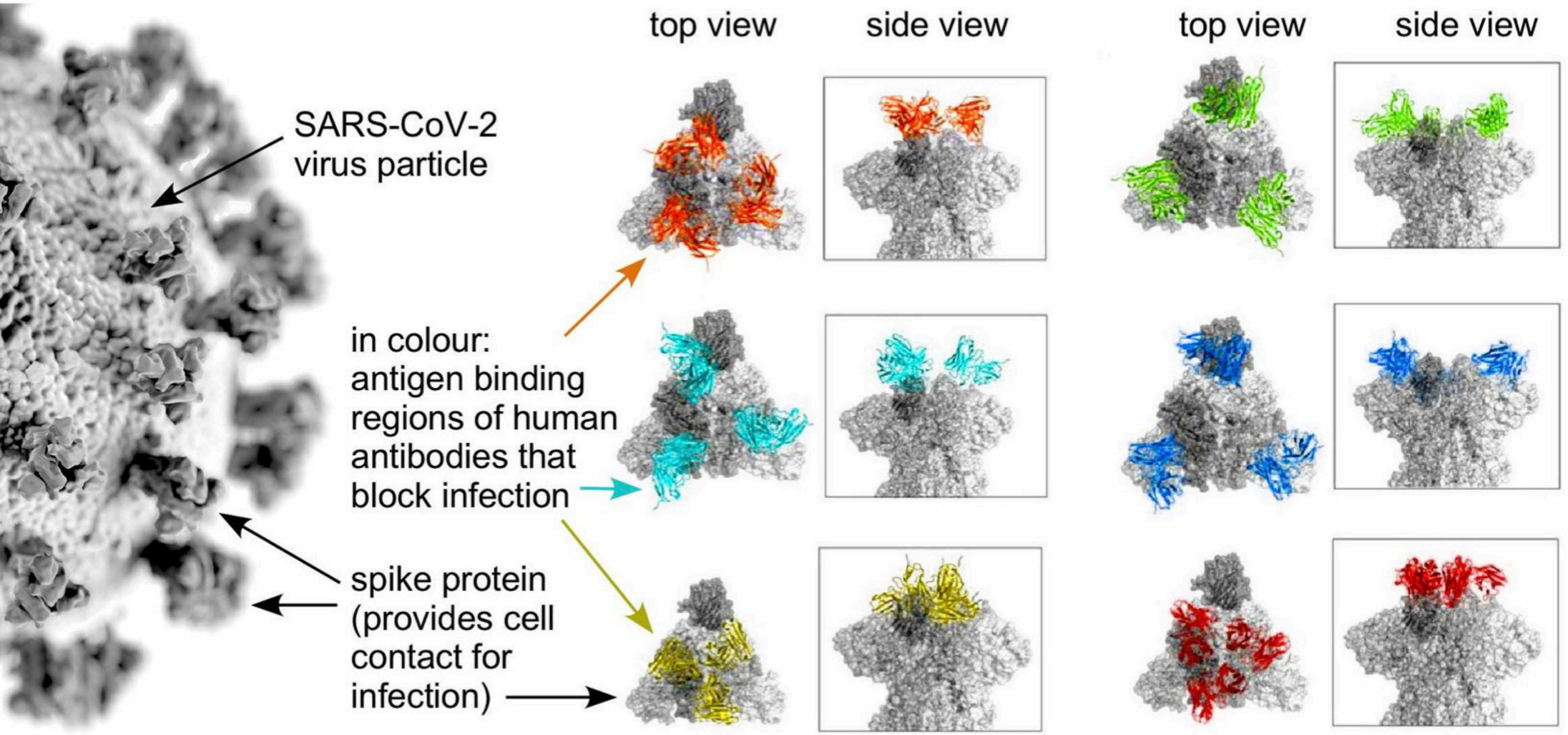
Antibodies efficiently blocking SARS-CoV-2



bioRxiv doi: 10.1101/2020.06.05.135921

Epitope Mapping: Biocopy/G. Roth, Modelling: Luca Varani et al.

SARS-CoV-2 Antibodies for diagnostics:
selected for no cross-reactivity with other corona viruses

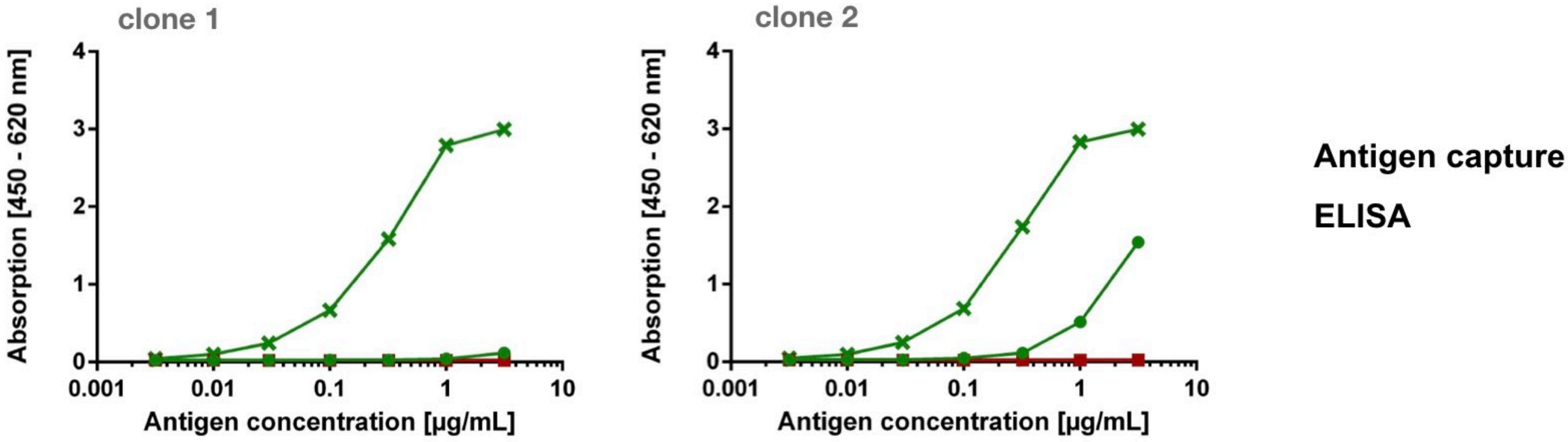


Plate immobilized anti-SARS-CoV-2 mIgG2a recAbs binding to SARS-CoV-2 Spike Glycoprotein S1 or HE domain from different human coronaviruses in ELISA. Mouse IgG2a recAbs anti-SARS-CoV-2 were immobilized in the wells of a 96-well plate at a concentration of 3.16 µg/mL (316 ng/well). Sino biological HIS-tag SARS-CoV-2 antigens (in green) or other human coronaviruses antigens (in red) were titered from a concentration of 3,16 µg/mL following a root of(10) dilution. Bound HIS-tag antigen was detected with HRP-conjugated anti-HIS antibody.

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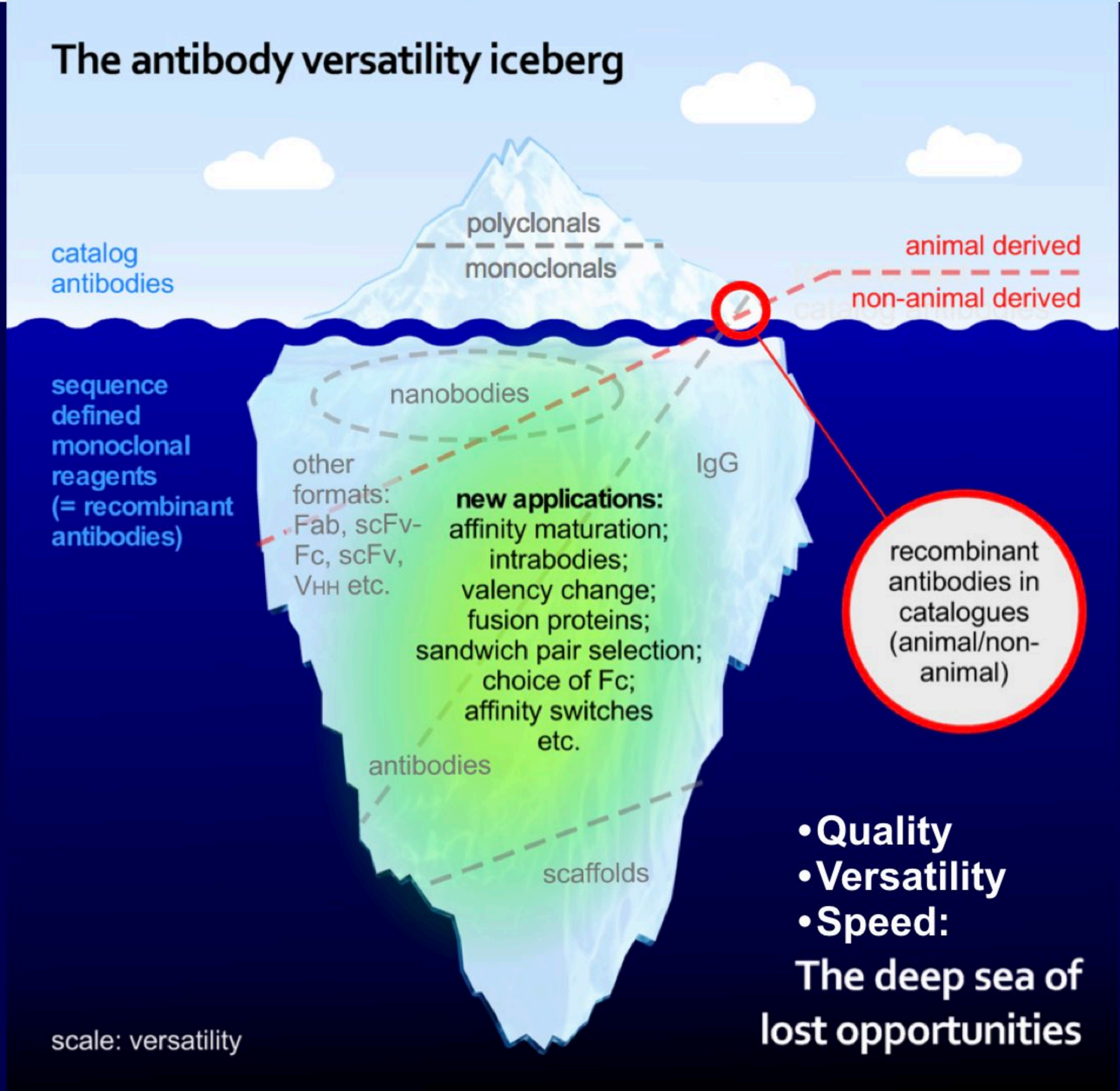
eXIST Existenzgründungen aus der Wissenschaft
ESF Europäischer Sozialfonds für Deutschland
Europäische Union

What we miss by not using more animal free antibodies in reserach



- **Quality**
- **Versatility**
- **Speed:**

The deep sea of lost opportunities

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Thanks to the people who did all the work!

& thanks to our many cooperators & funders:

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YUMAB THE ANTIBODY ENGINEERS

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DFG

SEVENTH FRAMEWORK PROGRAMME

SGC

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Abcalis (multiclonals)	hello@abcalis.com	abcalis.com