

SCIENTIFIC BENEFITS OF USING ANIMAL FRIENDLY AFFINITY REAGENTS (AFAS) AS REPLACEMENTS FOR ANIMAL-DERIVED ANTIBODIES.

Dr Alison Gray



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- 1.The traditional generation and use of antibodies, and associated problems
- 2.Overview and generation of AFAs, mechanistic principles mirroring nature
- 3.Benefits of animal free antibodies and affinity reagents
- 4.Barriers to their uptake
- 5.Sources of animal free technologies

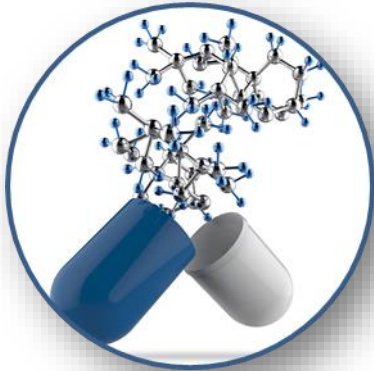


Mission (2013-present)

AFABILITY is striving to replace Animal Derived Antibody (ADA) production methods with Animal Friendly Affinity-reagents (AFAs) that do not require the use of animals, thereby significantly reducing the numbers and suffering of animals in the biomedical sciences and encouraging better science.

AFABILITY creates **awareness** surrounding the use of ADA production methods and alternatives by all scientific disciplines, challenges the **enforcement** of Directive 2010/63/EU and improves **accessibility** of replacement methods

ANTIBODY USE ACROSS ALL SCIENTIFIC DISCIPLINES



SOURCE	TARGET
Diagnostics and health	genetic testing, markers of infectious, chronic and sexually-transmitted disease, oncology
Therapeutics	rheumatoid arthritis, multiple sclerosis, Alzheimer's disease, Ebola and different types of cancers
Substances of abuse testing	MDMA, methadone, digoxin, cocaine, caffeine, ketamine, marijuana, methamphetamine and barbiturates
Anti doping programs for competitive sport	Erythropoietin, human growth hormone and human chorionic gonadotrophin
Fertility, ovulation and pregnancy testing	human chorionic gonadotrophin, luteinizing hormone, progesterone
Food safety and environmental contaminants in air, water, soil, packaging, processing / cooking and naturally occurring	Radionuclides, polycyclic aromatic hydrocarbons , arsenic, heavy metals, pesticides, natural toxins, lubricants, cleaning agents, carcinogens , Processing contaminants, hormones, veterinary drugs, allergens



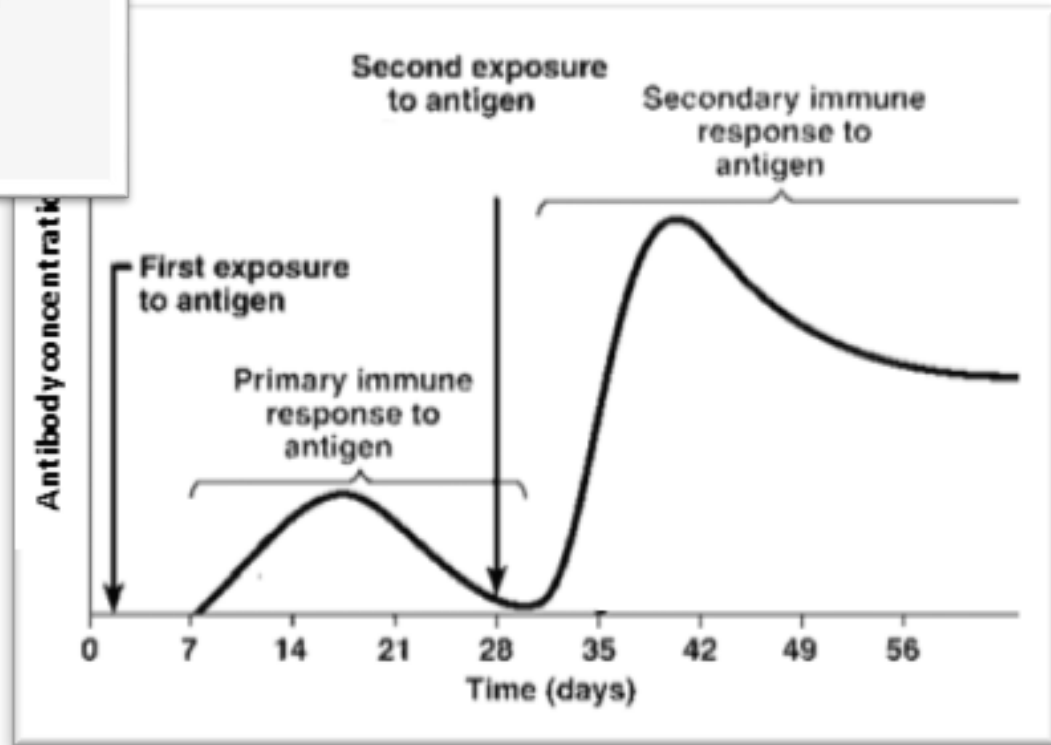
MAKING ANTIBODIES BY ANIMAL IMMUNIZATION...



Foreign particle / Antigen injected into animal....

Immune response to attack the intruder!

Extraction from the sera (blood) or B cells (spleen)



Estimated 1 million animals per year in EU (mice, rats, rabbits, ungulates, e.g., goats)

BEWARE OF USING ANIMAL DERIVED ANTIBODIES!



BEWARE OF USING ANIMAL DERIVED ANTIBODIES!



Polyclonal Antibodies (from serum) (Von Behring and Shibasaburo, 1898)

- 😊 inexpensive and quick to produce
- 😊 multiple epitope recognition
- 😞 high potential for cross-reactivity due to multiple epitope recognition and high background (only 0.5 - 5% positive in serum)
- 😞 batch-to-batch variability leads to irreproducible data
- 😞 not possible to store clone
- 😞 no easy way to determine the genetic sequences

Monoclonal Antibodies (from spleen) (Köhler and Milstein, 1975)

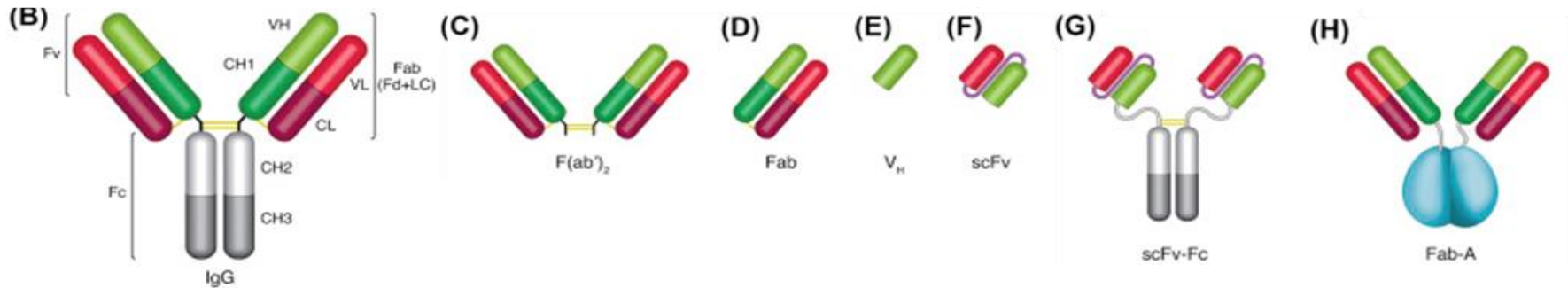
- 😊 More expensive and laborious to produce
- 😊 high specificity to only one unique epitope
- 😊 Assumed reduced probability of cross reactivity due to unique epitope recognition
- 😊 routinely achieve batch-to-batch homogeneity / finite reproducibility
- 😞 **Scientific literature littered with reports of non-specificity: a third of monoclonals had one or more additional productive binding sites (Bradbury *et al.*, 2018)**
- 😞 Can store but risk loss during storage / repeat use
- 😞 Possible to sequence but rarely done for R&D

....Resounding impact in terms of wasted cost, time and resources (annual loss of \$800m to the biomedical research community), as well as repercussions on diagnosis and health management.

ANIMAL FRIENDLY AFFINITY REAGENTS (AFAs)

Recombinant AFAs that are *non-animal-derived* / *non immunized*

- Antibodies



- Non antibody scaffolds or mimetics** (Aptamers, Affimers, DARPins, Affibodies, Monobodies, Anticalins and others)
- Production by naïve phage display (without animal immunization), yeast display or ribosome display etc

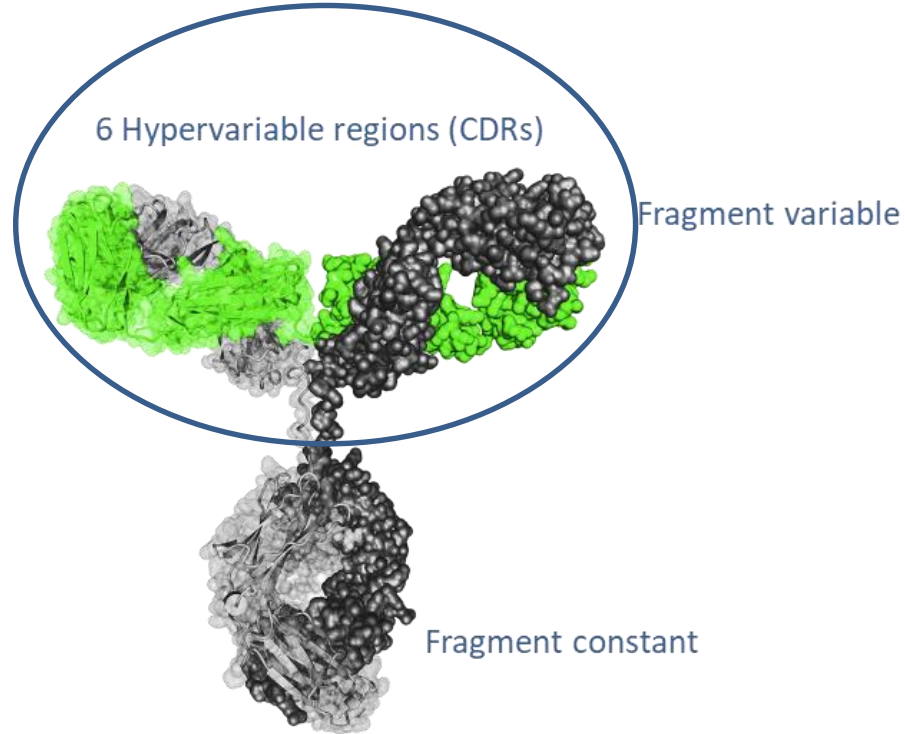
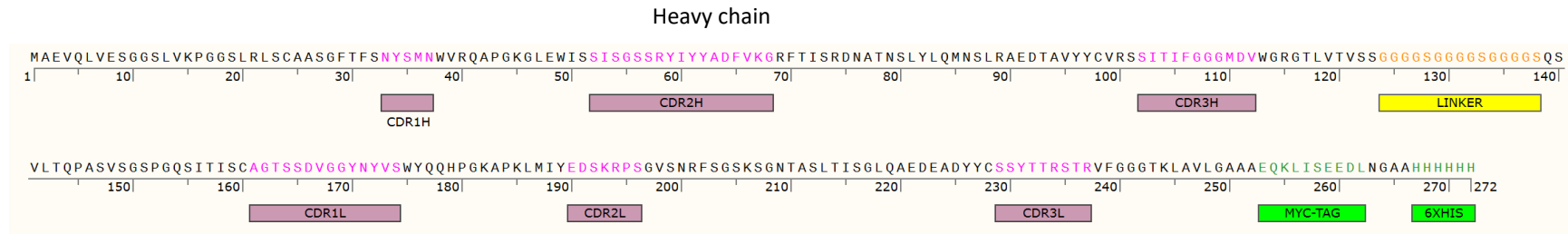
The 2018 Chemistry Laureates

The Royal Swedish Academy of Sciences has decided to award the Nobel Prize in Chemistry 2018 with one half to Frances H. Arnold "for the directed evolution of enzymes" and the other half jointly to George P. Smith and Sir Gregory P. Winter "for the phage display of peptides and antibodies".
[Read the press release](#)



Ill. Niklas Elmehed. © Nobel Media

AFA's: HARNESSING NATURAL DIVERSITY

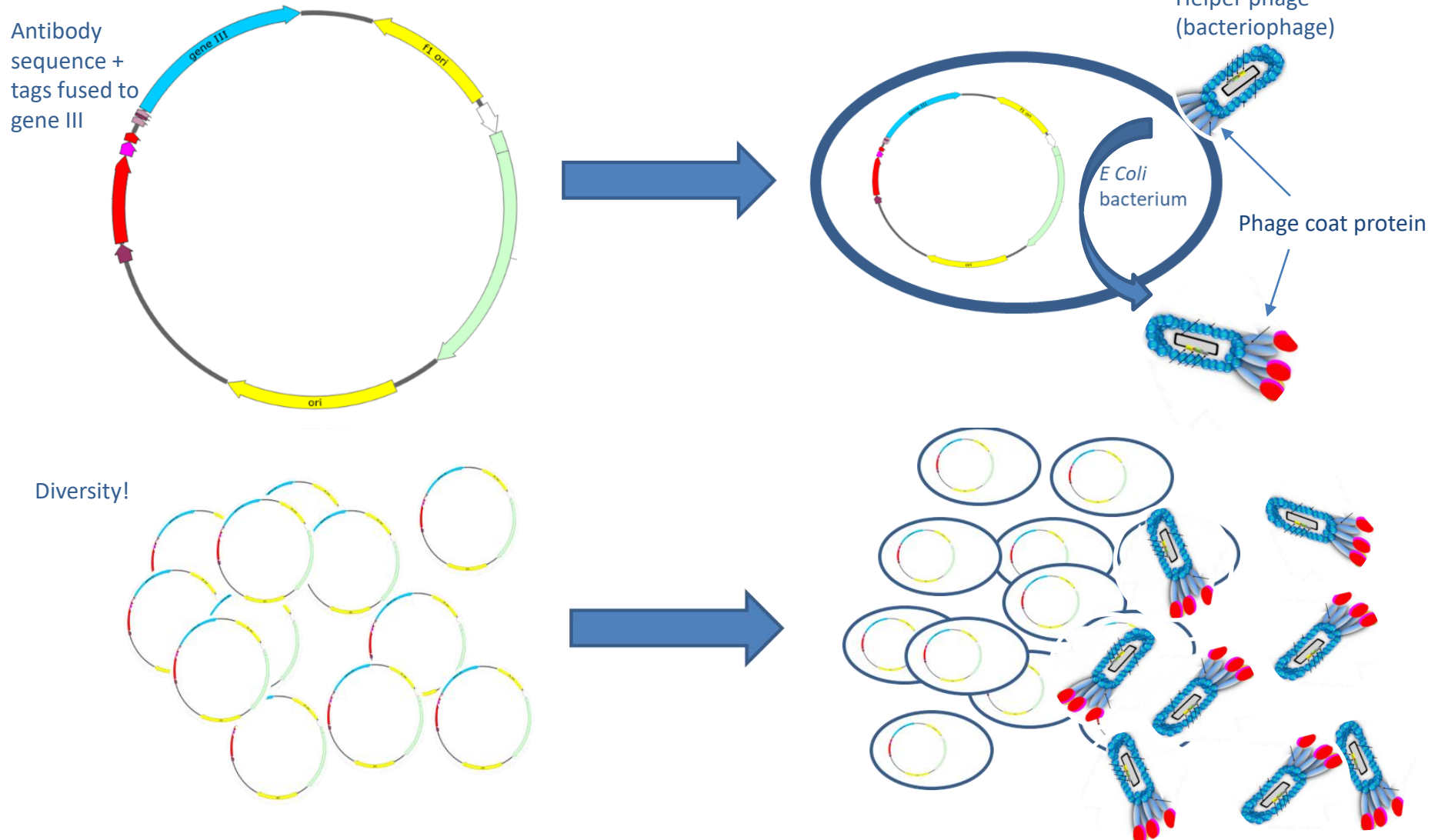


constructing the vector



..... or create diversity synthetically

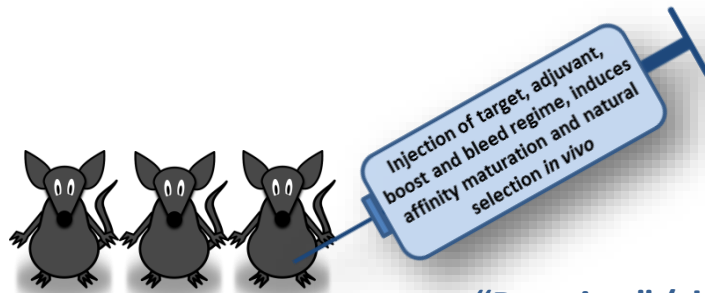
USING AFAs TO HARNESS NATURAL DIVERSITY: displaying the antibody



USING AFAs TO HARNESS NATURAL DIVERSITY: binder selection / surveillance

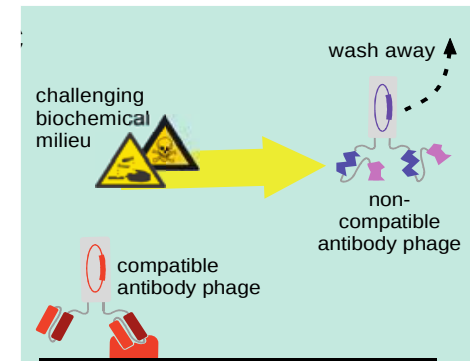
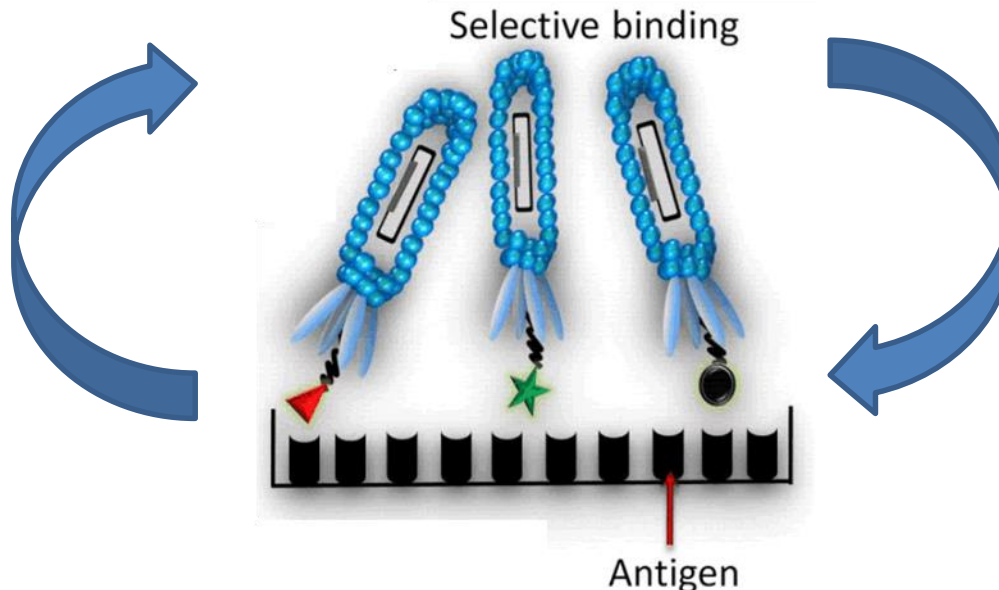
... these aren't instead of antibodies. These are antibodies!

AFAs adopt the same principles evolved by nature: mechanisms, repertoire / diversity, structure, surveillance, affinity, function...



“Panning” (the needle in the haystack)

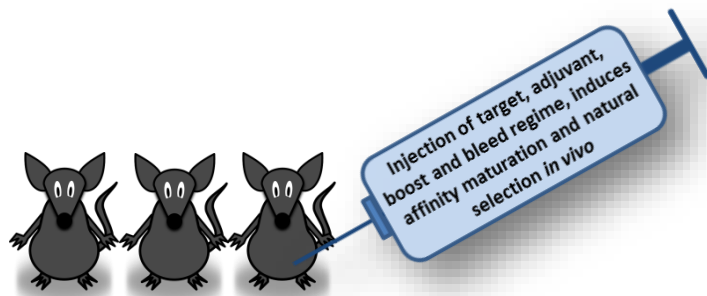
Consecutive panning and amplification cycles to give an increasingly enriched population of selective binders



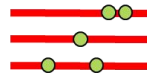
USING AFAs TO HARNESS NATURAL DIVERSITY: further refinement

... these aren't instead of antibodies. These are antibodies!

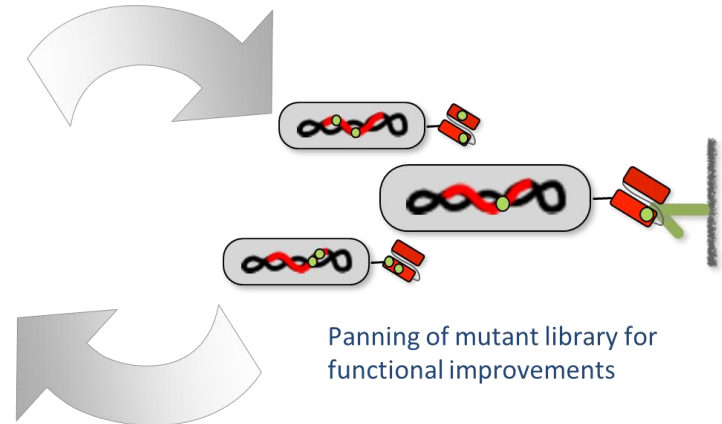
AFAs adopt the same principles evolved by nature: mechanisms, repertoire / diversity, structure, surveillance, affinity, function...



Affinity maturation (sculpted antibody)



Designed or PCR based
random mutagenesis or
variable chain shuffling as
diversity creating mechanisms



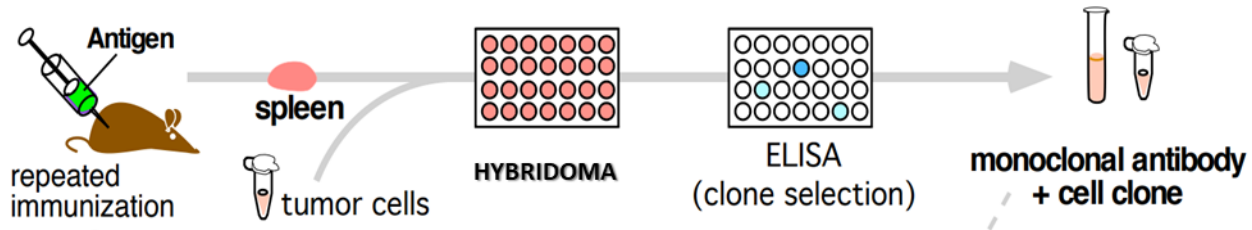
Panning of mutant library for
functional improvements

THE 3 MAIN ROUTES TO ANTIBODY PRODUCTION

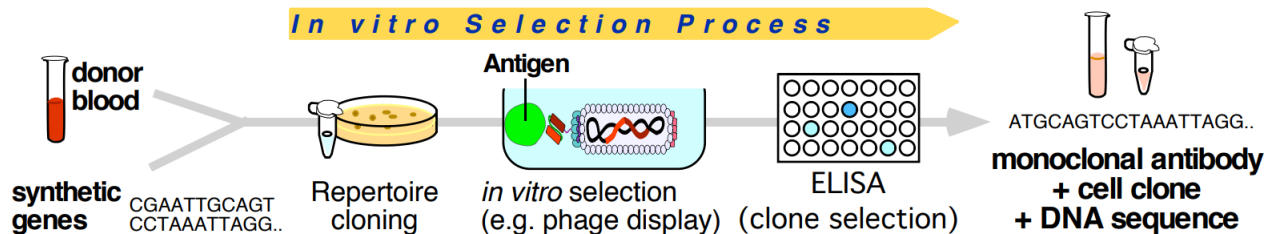
Polyclonal Antibodies (Antisera)



Monoclonal Antibodies from Hybridomas



Recombinant Antibodies



IMMUNIZED RECOMBINANT ANTIBODIES FROM HYBRIDOMAS / LIBRARIES

- Immunized (animal-derived) recombinant antibodies are also 'sequence defined' and have most of the same benefits as AFAs
- Costly and time consuming / no net reduction in animal use
- Immunization / new library required for each new antigen
- Suffer the same issues as monoclonals from hybridomas
- Be careful! Difficult to identify in catalogues

ADVANTAGES OF NON-ANIMAL-DERIVED ANTIBODIES

- The library is pre-designed with all desired features (capture and analysis tags, restriction sites etc)
- Antibody selection in ~ 4 weeks
- Opportunities for refinement: Selection under challenging conditions, Accessibility of gene sequences for downstream processing e.g., format changes, Next Generation Sequencing (NGS), affinity maturation
- One library equivalent to a life-time supply of animals
- AFAs are routinely sequence-defined from the start: Polyclonals not sequence-defined, monoclonals from hybridomas can be with additional effort
- You can always guarantee the reproducibility of your scientific results :
 - No batch to batch variation
 - No irretrievable loss of clone
 - No expensive storage conditions



Note: Certain scientific limitations common to both animal-derived and AFAs, due to the complexity of the natural antibody-antigen binding relationship: VALIDATE!

AFA_s AS POLYCLONALS

- A 'cocktail' of AFAs can be used to target multiple epitopes
- Multiclons (Abcalis™): a mixture of different, carefully selected AFAs with complementary epitope binding sites to amplify signal strength
- For applications where polyclonals have historically been relied upon, such as scorpion venom neutralization, it is also possible to neutralize the closely related toxins by using an AFA approach
- E.g., Diphtheria antitoxin: AFAs have replaced horse serum



WHY ARE WE NOT ALREADY USING REPLACEMENT METHODS?

- Scientific misconceptions block uptake of replacement methods including ...
 - Technology is not yet mature and ready to replace animals
 - Affinity is poor, need the whole animal response, require immunized animals

AfAs for research in EU consortia

Project	AfA clones (from phage display)	against # of antigens
Affinity Proteome	130	48
Affinomix	2,151	582
Antibody Factory	461	204
Sanger Atlas	7,236	292
SGC-Pilot	340	20
total	103,181	1,146

total: incl. 193 Darpins

Sources: <https://cordis.europa.eu>, Schofield et al. 2007, Colwill et al. 2011



AfAs for therapeutics

INN	Trade name	Developer	Library	Target	Indication	Status ¹
Ramucirumab	Cyramza	Eli Lilly	Dyax	VEGFR2	NSCLC, colorectal cancer, gastric cancer	Approved
Belimumab	Benlysta	HGS/GSK	CAT	BLyS	Systemic lupus	Approved
Raxibacumab	ABthrax	HGS/GSK	CAT	B. anthracis	Prophylaxis and treatment of anthrax	Approved
Necitumumab	Portrazza	Lilly/BMS	Dyax	EGFR	Squamous lung cancer	Approved
Bimagrumab		Novartis	Morphosys	Act1R	Sporadic inclusion body myocytis	Phase III
Tralokinumab		Astra Zeneca	CAT	IL-13	Asthma, ulcerative colitis	Phase III
Gantenerumab		Roche	Morphosys	Amyloid beta	Alzheimer's disease	Phase III
Guselkumab	Tremfya	Janssen	Morphosys	IL-23p19	Plaque psoriasis	Phase III
Avelumab	Bavencio	Merck KGaA/Pfizer	Morphosys	PD-L1	NSCLC	Phase III

WHY ARE WE NOT ALREADY USING REPLACEMENT METHODS?

(addressed by the ESAC report / ECVAM recommendation)

- Scientific misconceptions block uptake of non-animal-derived antibodies including ...
 - Technology is not yet mature and ready to replace animals
 - Affinity is poor, need the whole animal response, require immunized animals
 - Loss of polyclonal characteristics
- Lack of understanding of the new, sophisticated technology...shrouded in mystery!
- Lack of expertise / motivation by users of traditional methods and a propensity toward familiar methods due to economics /time factor / implementation / inertia
- Focus on pharma, high prices, royalties
- Accessibility to commercial AFAs limited (but improving) ...

ACADEMIC SUPPLIERS OF AFAS

The image displays three overlapping screenshots of academic websites, each representing a supplier of antibodies for AFAS.

- TPAC Toronto Recombinant:** The top-left screenshot shows the TPAC logo (a stylized blue 'Y' shape) and the text "TPAC Toronto Recombinant". Below the logo, it says "WELCOME TO THE TORONTO" and "Antibodies have emerged as the major...".
- UNIVERSITÉ DE GENÈVE GENEVA ANTIBODY FACILITY:** The middle screenshot shows the Université de Genève logo and the text "GENEVA ANTIBODY FACILITY". It features a navigation bar with "Home", "Antibody Discovery", and "Antibody Production". Below this, there are two large purple circles with the text "Antibody Discovery" and "Antibody Production".
- IPI Protein Innovation Antibody Initiative:** The bottom-right screenshot shows the IPI Protein Innovation logo and the text "Antibody Initiative". It features a background image of a protein structure. Below the header, there is a "Strategy" section with a list of links: "Platform", "Nominations", and "Future Initiatives".

...Warning: Others are not as 'animal-free' as they claim...

AFAS FROM COMMERCIAL SOURCES

The image displays a collage of five commercial antibody provider websites, illustrating various sources for animal-free antibodies (AFAS).

- BIO-RAD:** Features a navigation menu with 'Products' and 'Custom Services'. The 'Custom Services' section lists options like 'Custom Antibody Services', 'Recombinant Antibody Generation', 'Bulk Catalog Antibody Production', 'Antibody Conjugation Service', 'Antibody Conjugation Kits', 'Custom Services Inquiry Form', and 'ISO 9001 Quality Assurance Management'.
- abcam:** Includes a search bar with the placeholder 'enter keyword e.g., p53, western blot or abID product code' and a 'Research Products' section.
- AdipoGen® LIFE SCIENCES insights:** Promotes 'Recombinant RecMAbs™' and states: 'AdipoGen Life Sciences (AdipoGen) To promote the advancement of phage display technology in-house to develop AdipoGen was a forerunner in releasing 2010: anti-APRIL (mouse), mAb (human) antibodies made with the phage display technology to block or activate their respective targets.' It also mentions: 'Our phage display relies on a large library of genetic information and the unique phenotypes. We make use of are M13 phagemid libraries with complementary determining regions (CDRs) from parental clones in the library to less than 1%.' A small text box at the bottom left reads: 'Wikipedia: 3rd biggest research antibody provider'.
- absolute antibody:** Features a search bar with the placeholder 'Search for antibodies...' and a navigation menu with 'HOME' and 'CORE TECHNOLOGIES'. It highlights 'Animal-Free Antibodies' and 'Recombinant Versions of Natural Antibodies'.
- Abcalis:** Promotes 'A new Class of Antibodies. Animal-Free. Sequence Defined.' and includes a navigation menu with 'Home', 'Mission', 'Products', 'Contact', and 'Impressum'. It features a large image of a forest and a microscopic view of cells. The 'Animal Use Statement' section states: 'Abcalis has the vision to generate and produce antibodies entirely without animal experiments. We achieve this by using the animal free method of *in vitro* selection by phage display from antibody gene libraries to generate monoclonal antibodies. Abcalis does not generate hybridomas. Moreover, in the production process, our cultivation media are free of animal derived materials, like fetal calf serum or BSA. Abcalis antibodies may contain animal derived sequences. Examples are the genes encoding the mouse Fc, rabbit Fc or other animal Fc parts of Abcalis antibodies. The use of these constant region sequences is unavoidable to provide compatibility to our customers' applications. Abcalis did not use any laboratory animals to obtain these sequences, which were chemically synthesized based on publications or obtained as recombinant DNA from commercial sources.' It also includes buttons for 'products' and 'shop now'.

**Thank
you!**

