Technology Networks

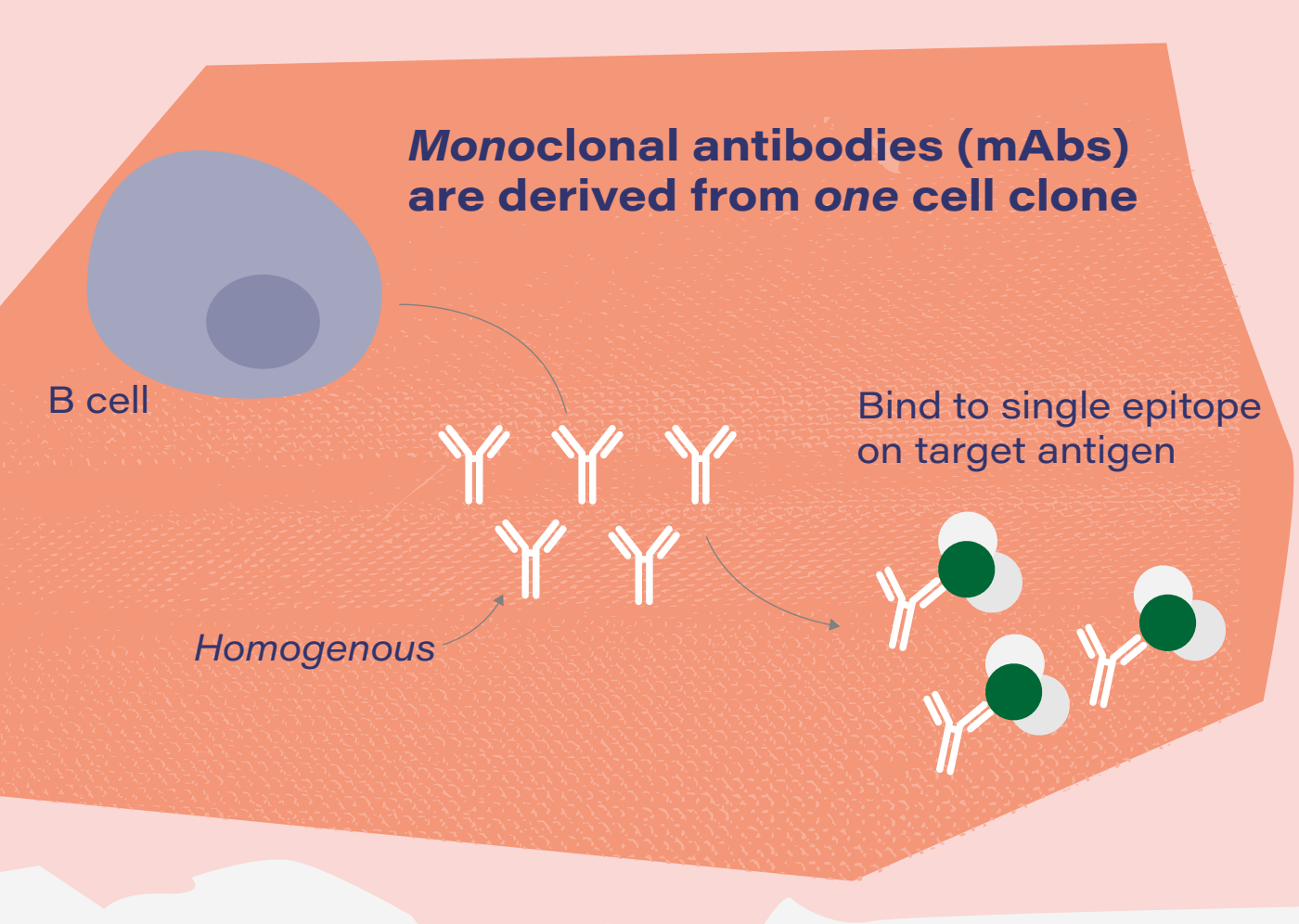
THERAPEUTIC ANTIBODIES

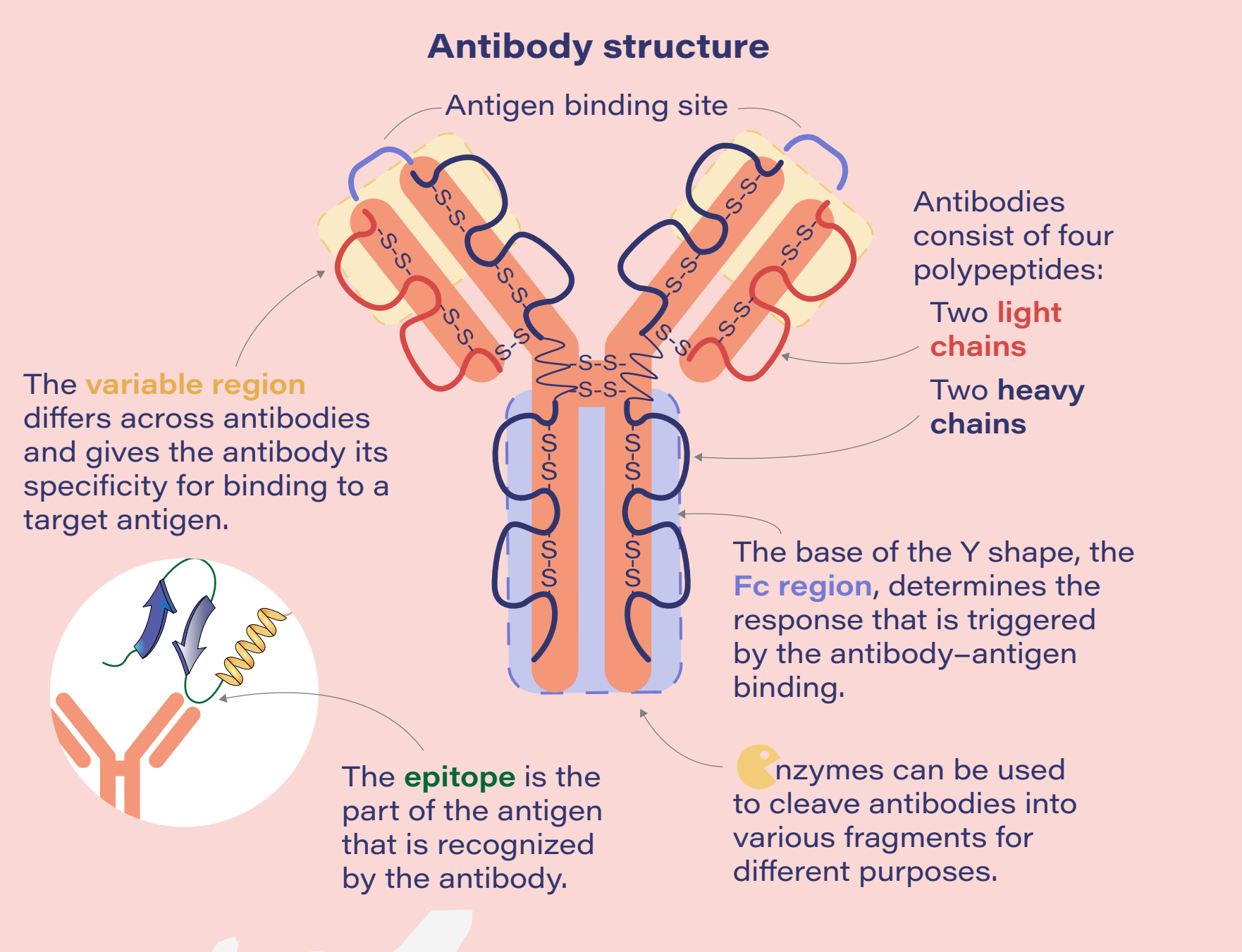
This infographic will explore advances related to the research and development of therapeutic antibodies for different disease applications.

WHAT ARE ANTIBODIES?

Antibodies (Abs) are Y-shaped glycoproteins that are produced by B lymphocytes in response to the presence of a foreign molecule, like a virus.

Antibodies recognize and bind to antigens that are presented by the foreign molecule, triggering an immune response that neutralizes the pathogen.

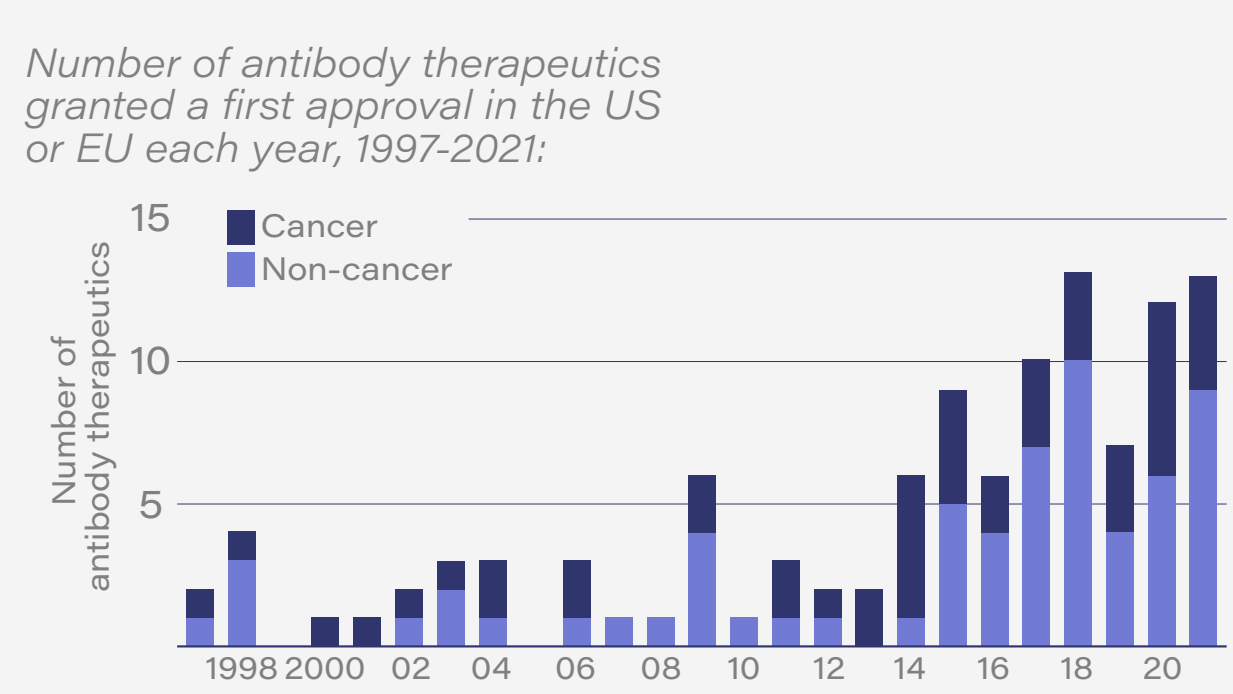




THERAPEUTIC MABS

Antibodies bind their target antigen with high specificity. Researchers have leveraged this specificity to create antibodies that can be used as drugs to treat a variety of human diseases.

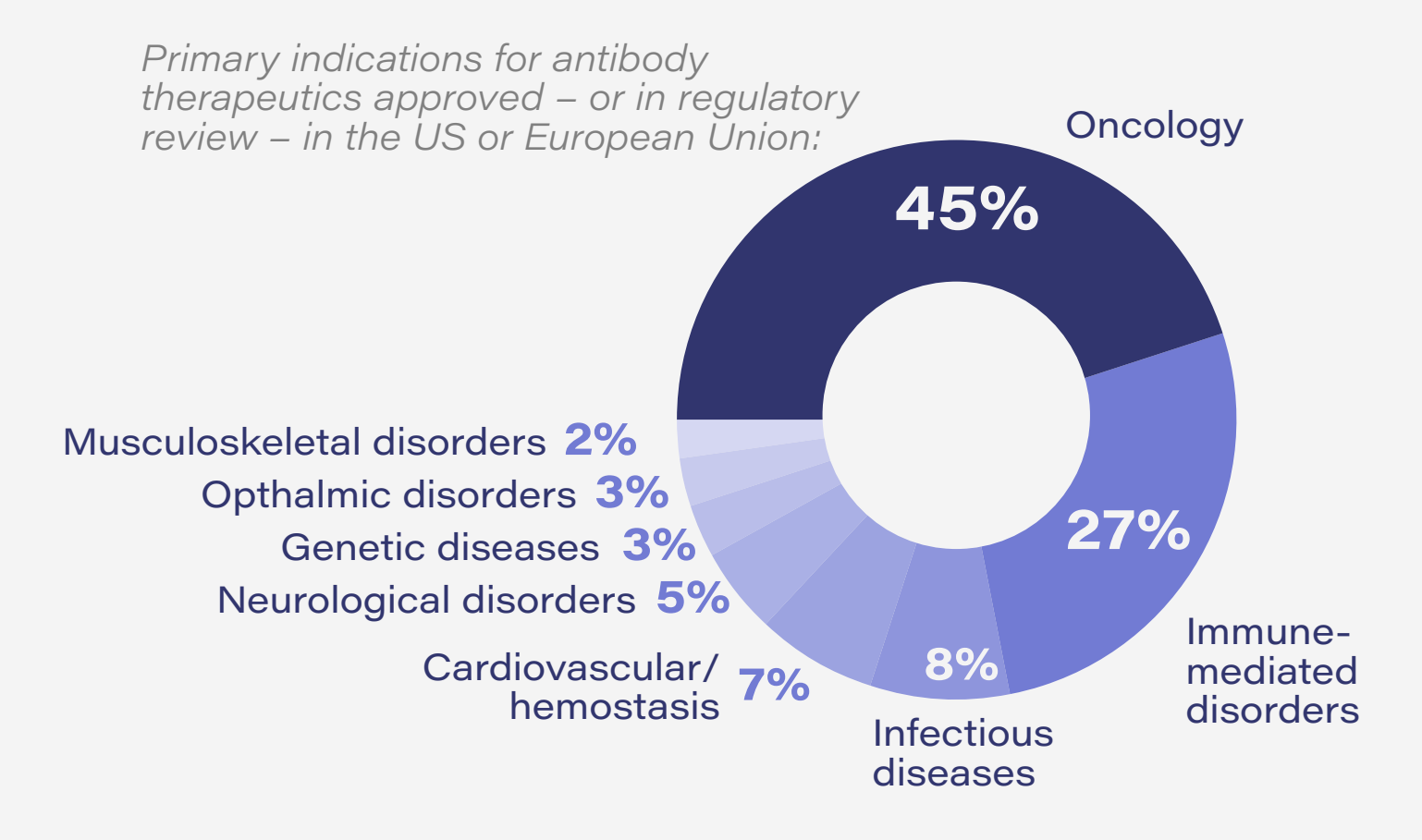
Number of antibody therapeutics granted a first approval in the US or EU each year, 1997-2021:



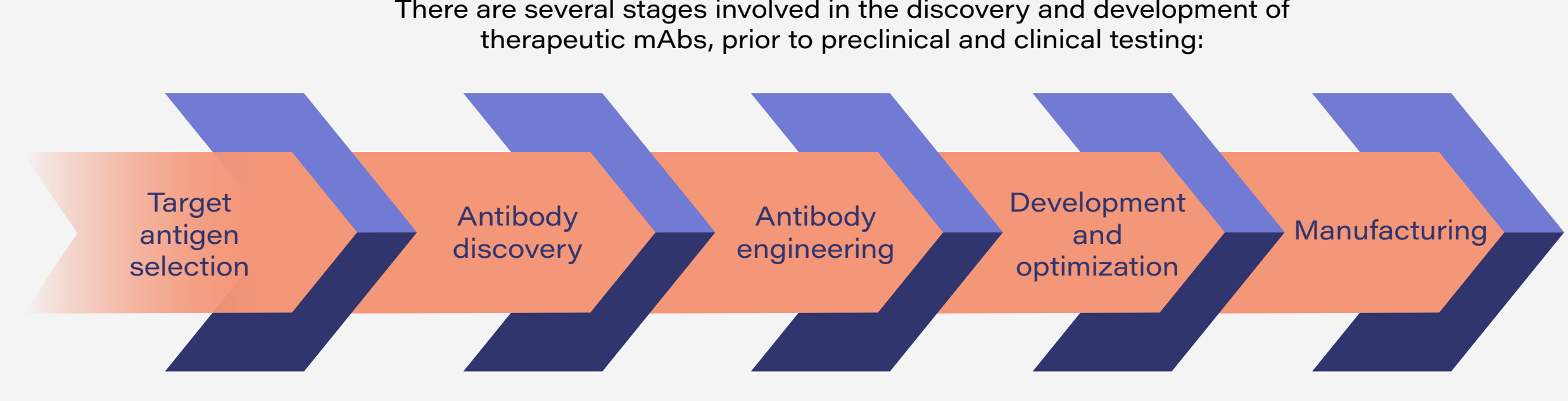
Source: The Antibody Society

While the majority of therapeutic antibodies approved or in regulatory review are indicated for cancer treatment, other disease areas are being targeted too, such as:

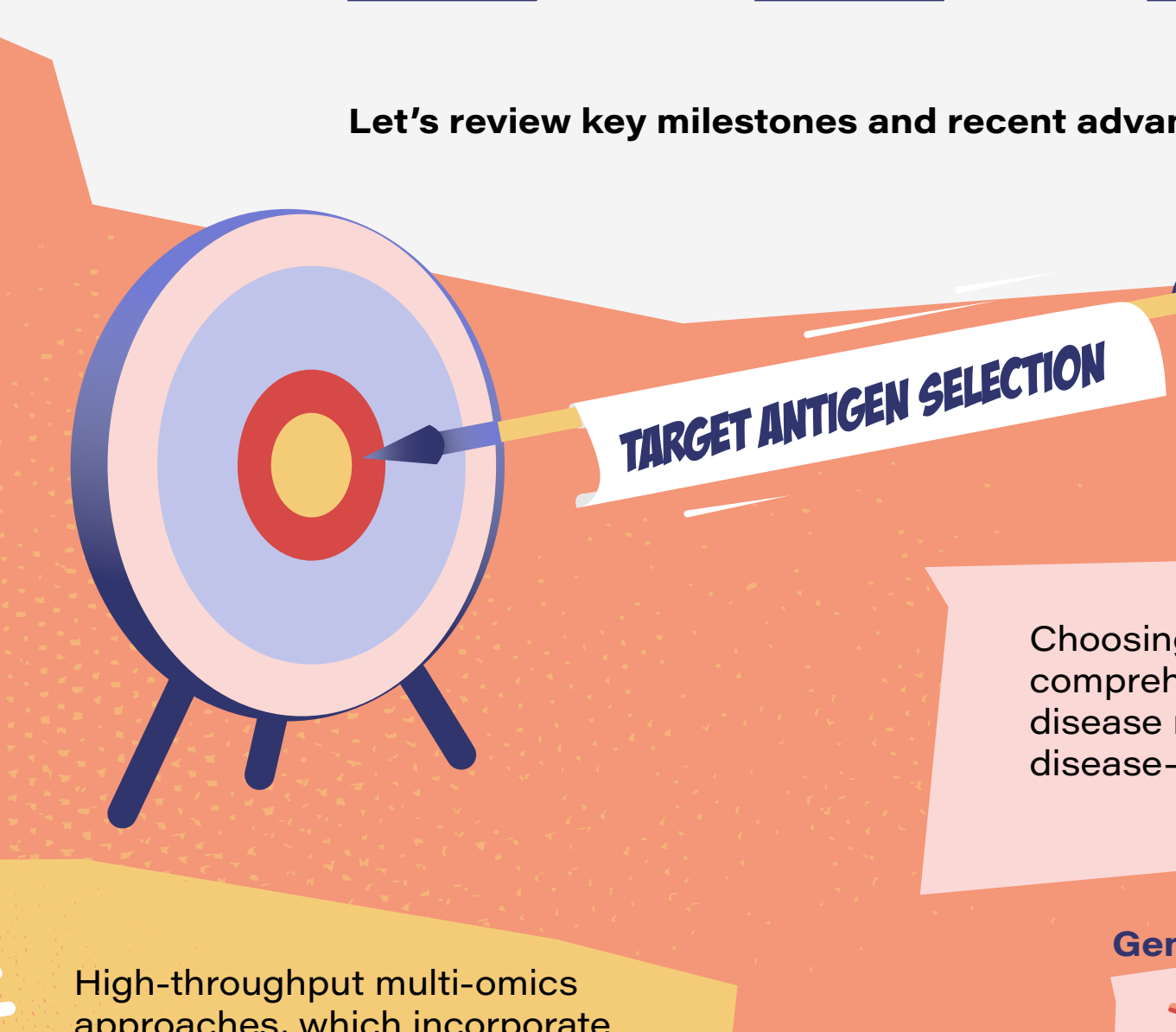
Primary indications for antibody therapeutics approved – or in regulatory review – in the US or European Union:



There are several stages involved in the discovery and development of therapeutic mAbs, prior to preclinical and clinical testing:

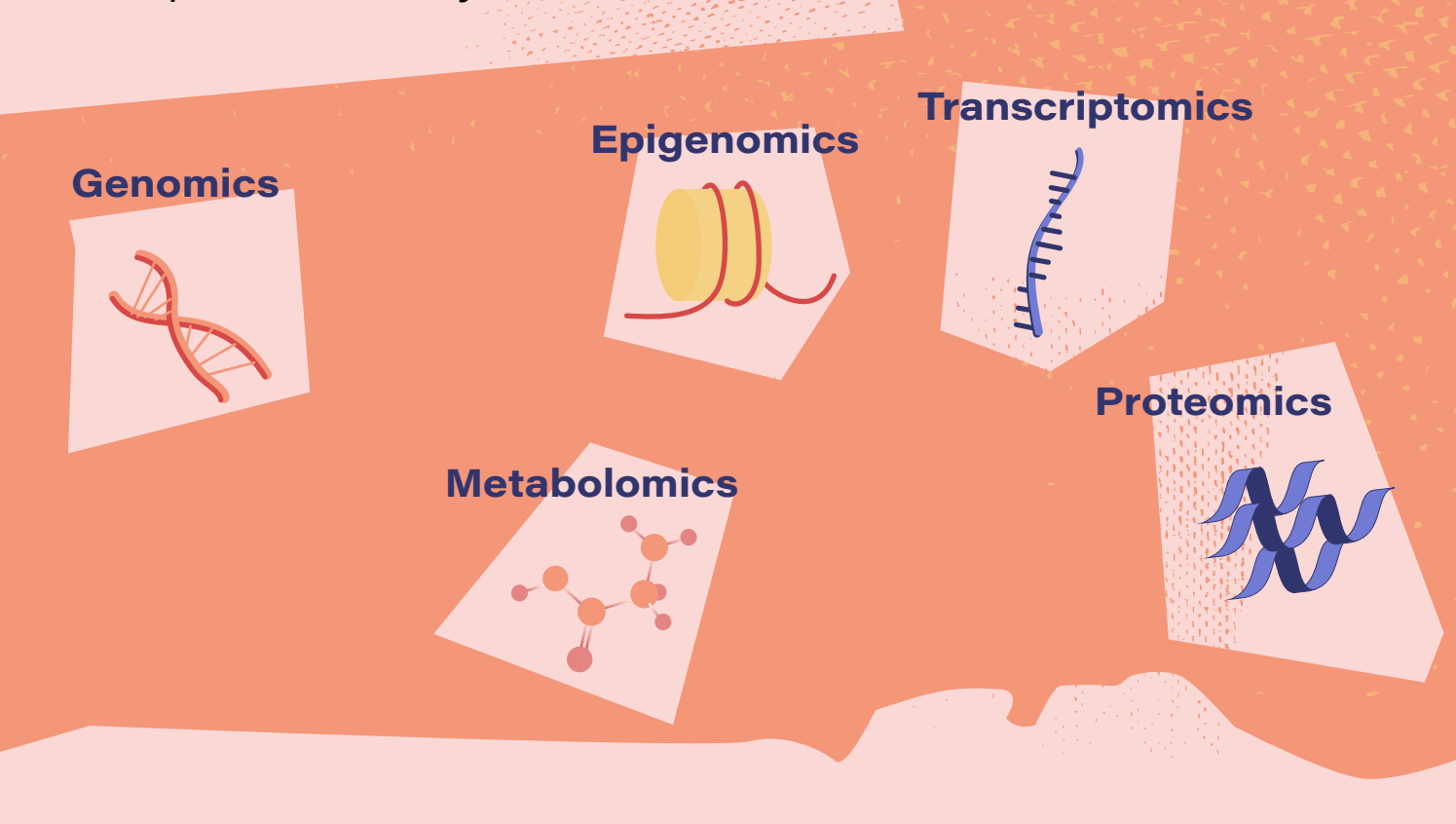


Let's review key milestones and recent advancements in various stages of this pipeline.



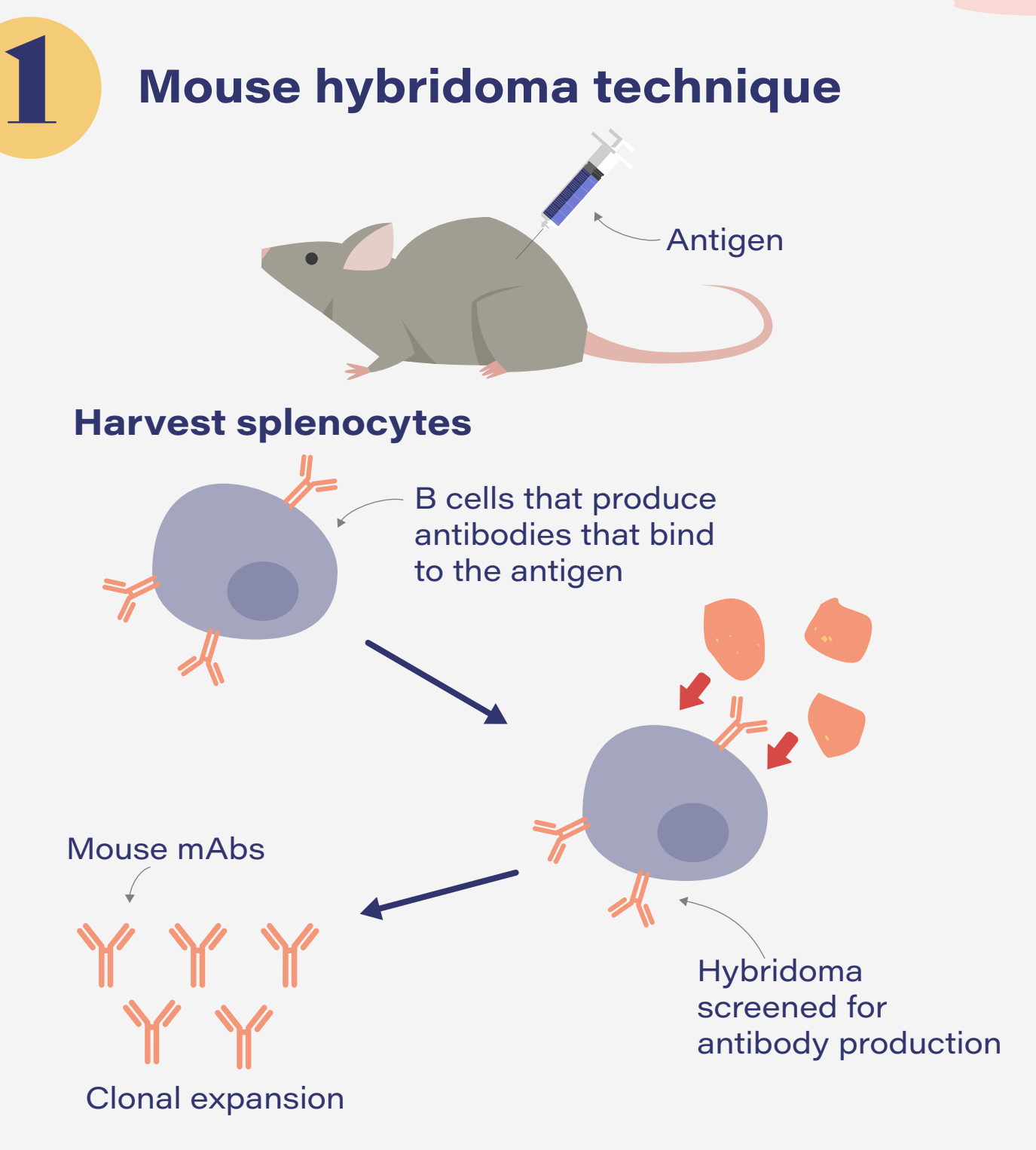
Choosing a target antigen requires comprehensive understanding of a disease mechanism, or a disease-specific antibody effect.

High-throughput multi-omics approaches, which incorporate analysis of the genome, epigenome, transcriptome, proteome and metabolome, can now offer holistic insights into disease pathology – at a single-cell level, in some cases.



KEY ADVANCEMENTS THAT HAVE REVOLUTIONIZED ANTIBODY DISCOVERY AND ENGINEERING

1 Mouse hybridoma technique



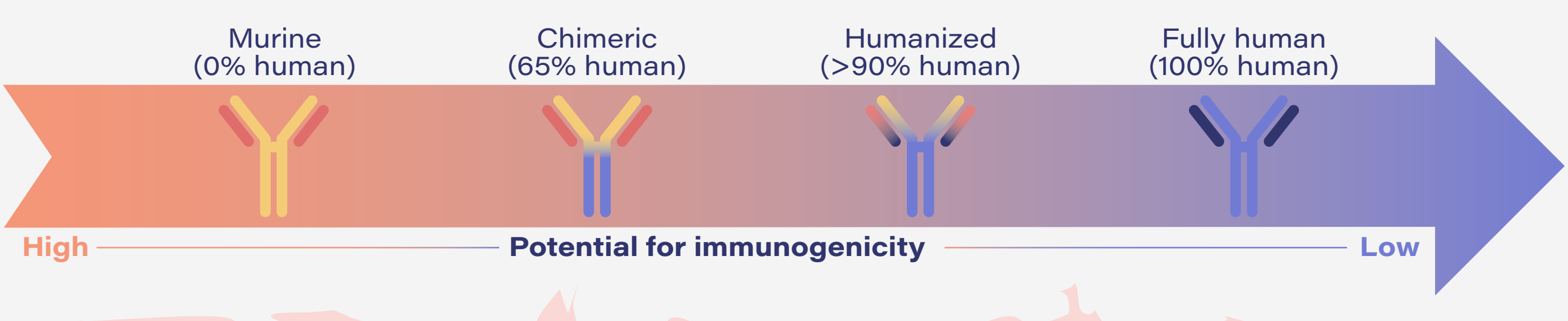
1975 The seminal hybridoma technique developed by Köhler and Milstein in 1975 shaped the field of antibody development for therapeutic applications.

However, mouse mAbs can trigger adverse reactions in humans, such as the "human anti-mouse antibody" (HAMA) response, limiting their utility.

1980s Non-human variable domains were combined with human constant domains to manufacture antibodies with 65% human content, a method known as chimerization.

1997 Rituximab (Rituxan®) – the first chimeric therapeutic antibody – was approved in 1997 for the treatment of cancer.

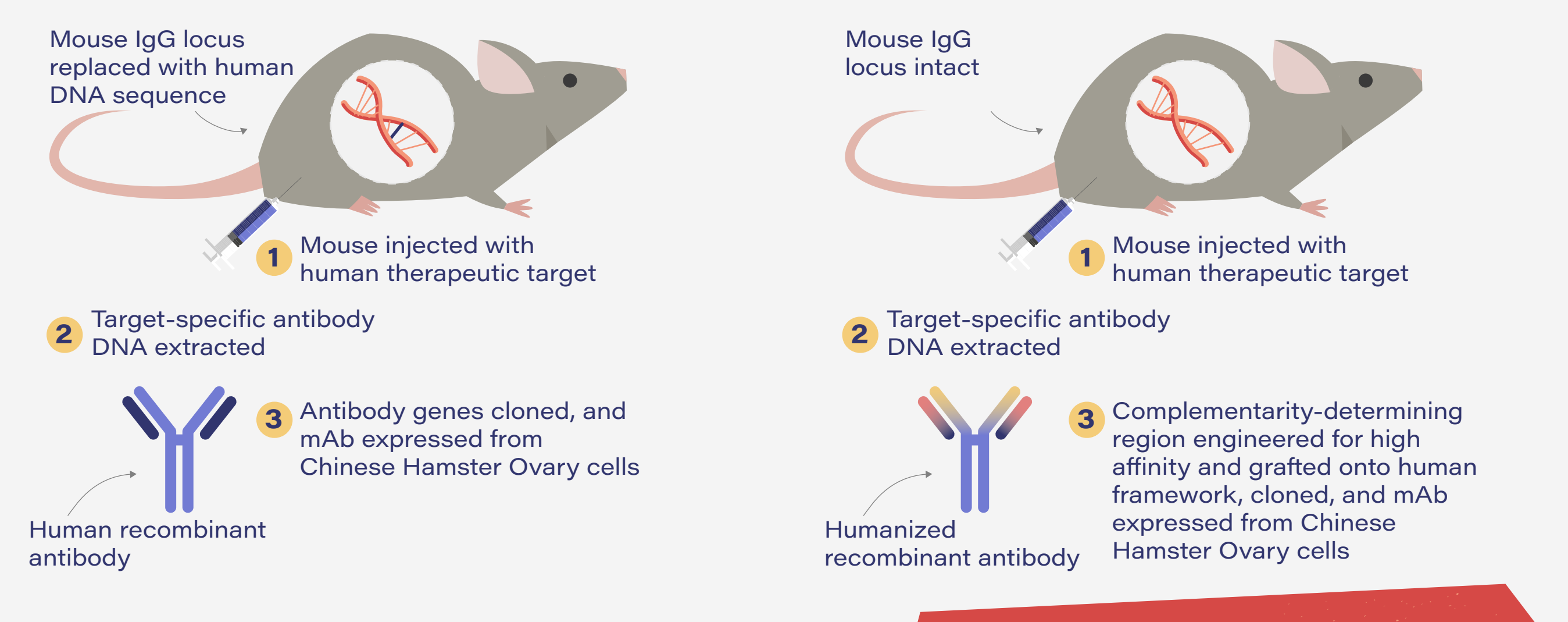
1980s-present Over the last few decades, novel technology platforms emerged and have been refined to produce increasingly human-like mAbs (or humanized).



2 Transgenic mice engineered to produce human antibodies

A "breakthrough" for therapeutic mAb development

Transgenic mice have been created that can produce humanized or completely human antibodies:



Owing to advances in next-generation sequencing, we can now sequence the genes encoding the entire antibody repertoire of an animal.

With this knowledge, scientists are able to explore the effects of manipulating this repertoire and discover novel antibodies that may exist in human populations.

> "These advancements will no doubt allow the recovery of rare antibodies with properties satisfying the demanding design goals of the next generation of therapeutic targets"
> **Chen and Murawsky**

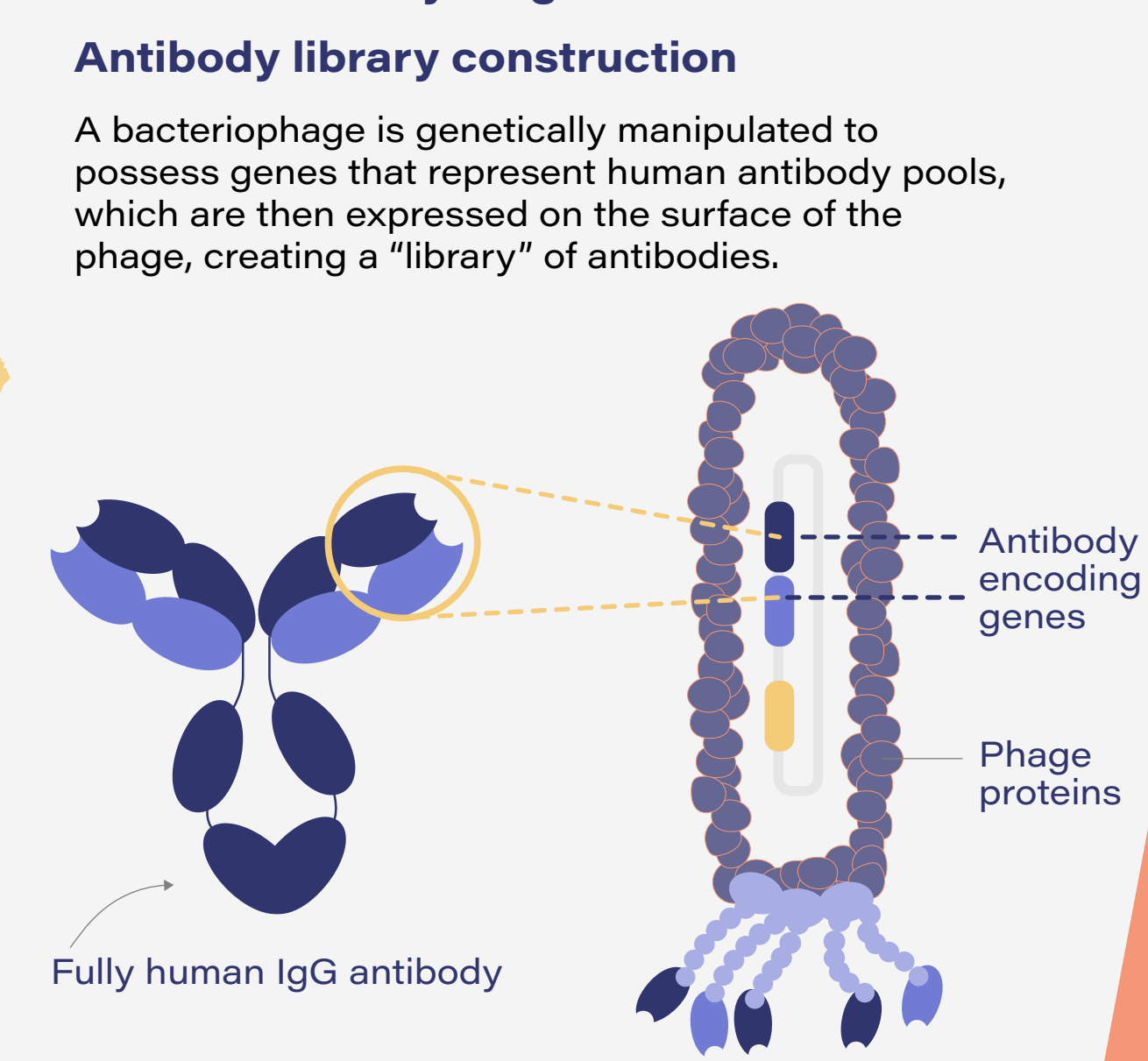
3 Phage display

The most widely used *in vitro* approach for *de novo* generation of antibody candidates

Involves two key stages:

Antibody library construction

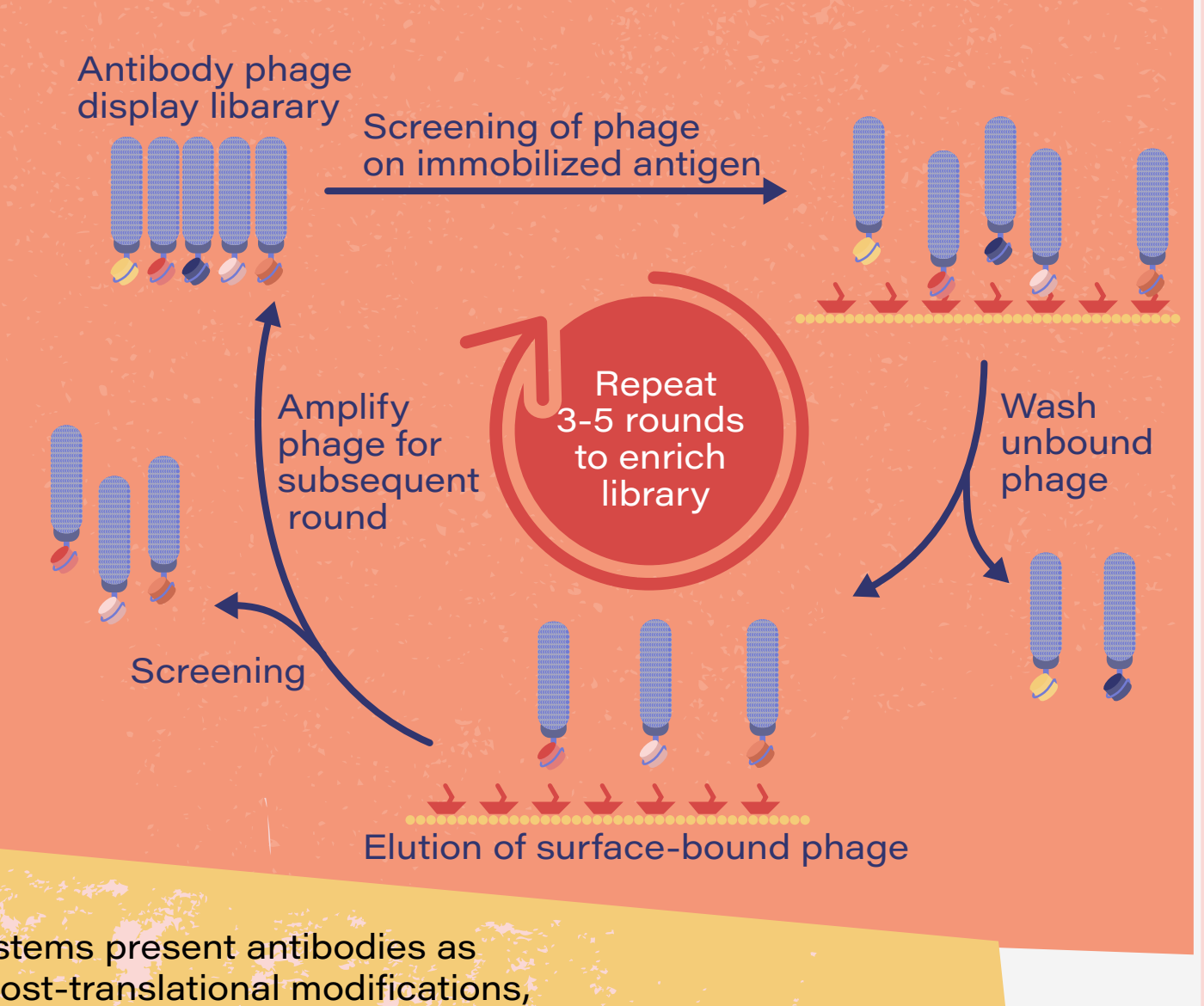
A bacteriophage is genetically manipulated to possess genes that represent human antibody pools, which are then expressed on the surface of the phage, creating a "library" of antibodies.



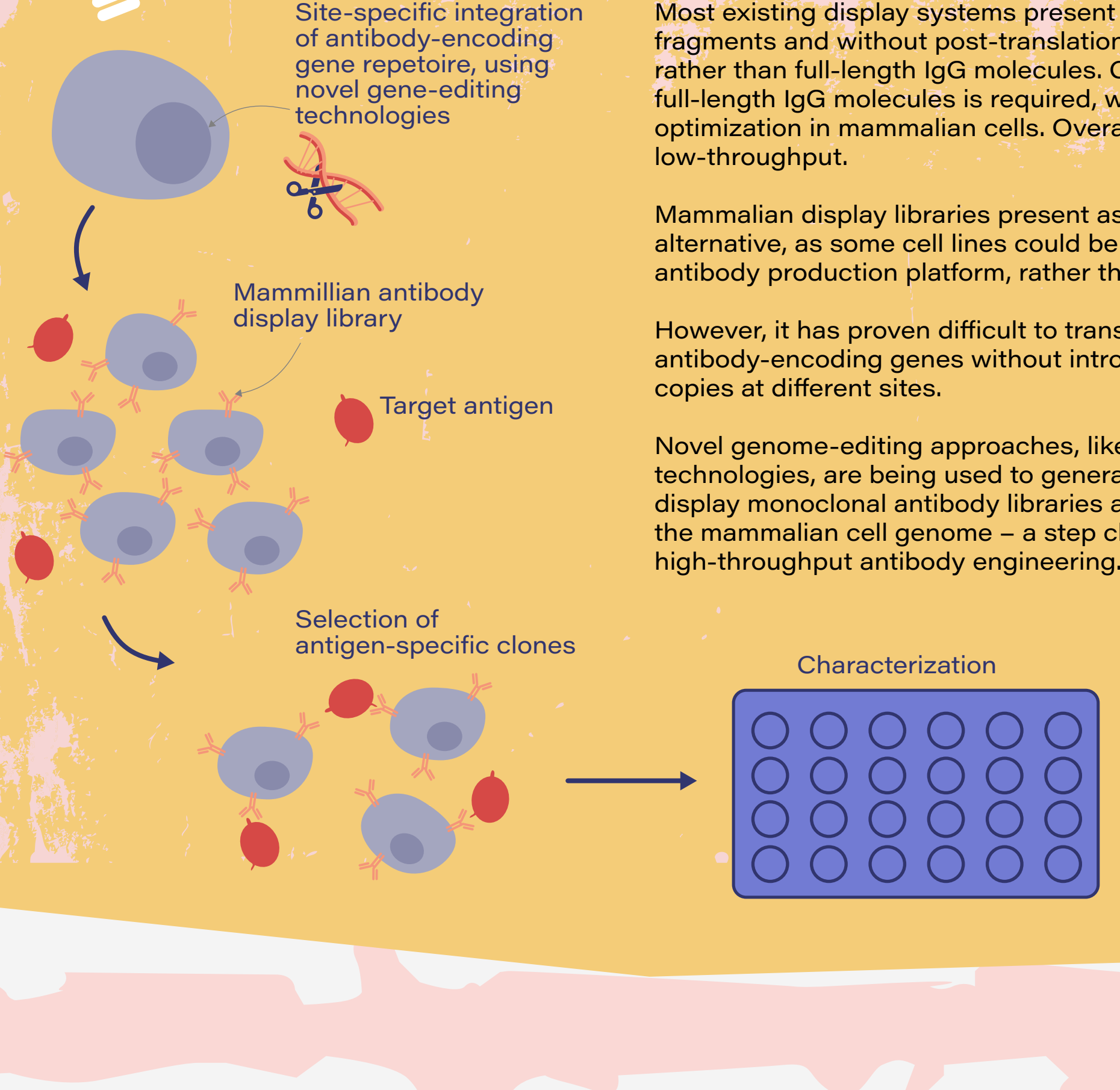
Biopanning

The phage library is incubated with the immobilized antigen of interest.

Unbound phage are then removed via multiple rounds of washing, and bound phages are eluted, amplified and the process repeats for several rounds to enrich the antibody population that binds to the antigen of interest.



Advancement



Most existing display systems present antibodies as fragments and without post-translational modifications, rather than full-length IgG molecules. Conversion to full-length IgG molecules is required, with subsequent optimization in mammalian cells. Overall, this process is low-throughput.

Mammalian display libraries present as a potential alternative, as some cell lines could be directly used as the antibody production platform, rather than just as a library.

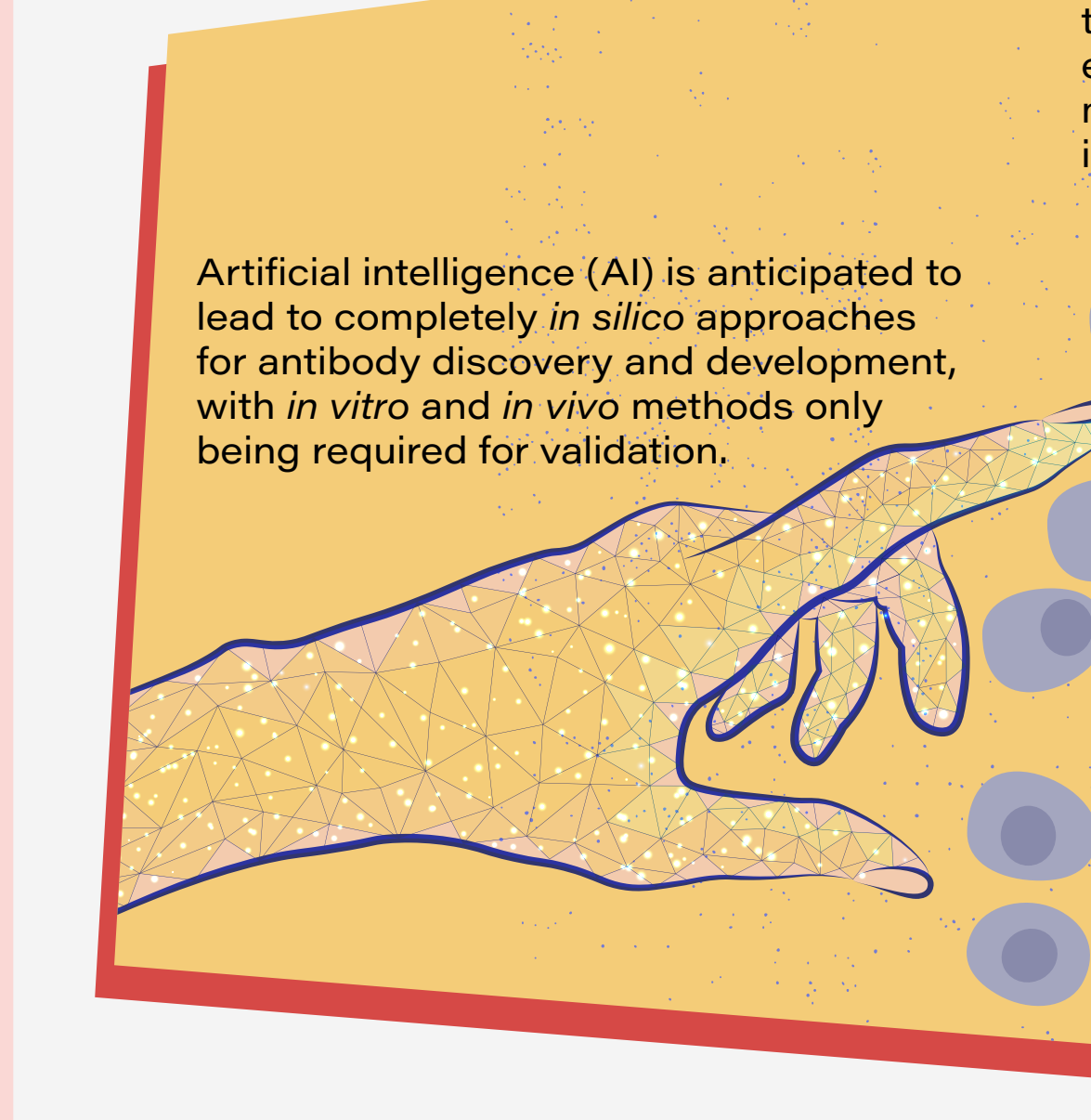
However, it has proven difficult to transfect antibody-encoding genes without introducing multiple copies at different sites.

Novel genome-editing approaches, like CRISPR-Cas9 technologies, are being used to generate increasingly larger display monoclonal antibody libraries at pre-defined sites in the mammalian cell genome – a step closer to high-throughput antibody engineering.

For several mammalian systems, the library cells can be used directly for antibody production and chaterization.

4 Sourcing mAbs from human cells

Relies on the robustness of the human immune system.



Peripheral blood mononuclear cells (PBMCs) are isolated from humans that have been infected with a pathogen or vaccinated.

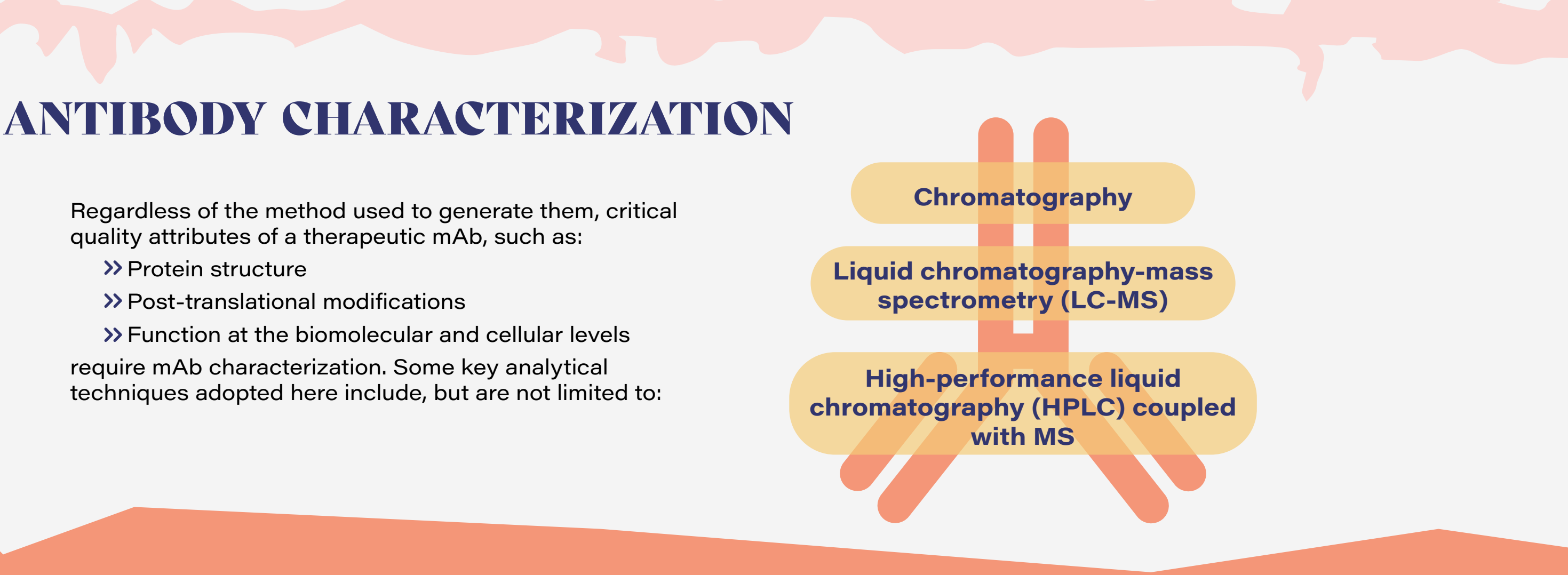
Flow cytometry is utilized to sort cells based on their cell surface expression markers.

After B cells are isolated, immunoglobulin transcripts are amplified using reverse transcriptase polymerase chain reaction (RT-PCR).

Gene transcripts are cloned and expressed in mammalian cell lines to generate recombinant mAbs.

The advancement of high-throughput technologies to rapidly sort B cells provides the opportunity to efficiently study antibody repertoires and develop mAbs for public health emergencies, such as infectious diseases.

Artificial intelligence (AI) is anticipated to lead to completely *in silico* approaches for antibody discovery and development, with *in vitro* and *in vivo* methods only being required for validation.



ANTIBODY CHARACTERIZATION

Regardless of the method used to generate them, critical quality attributes of a therapeutic mAb, such as:

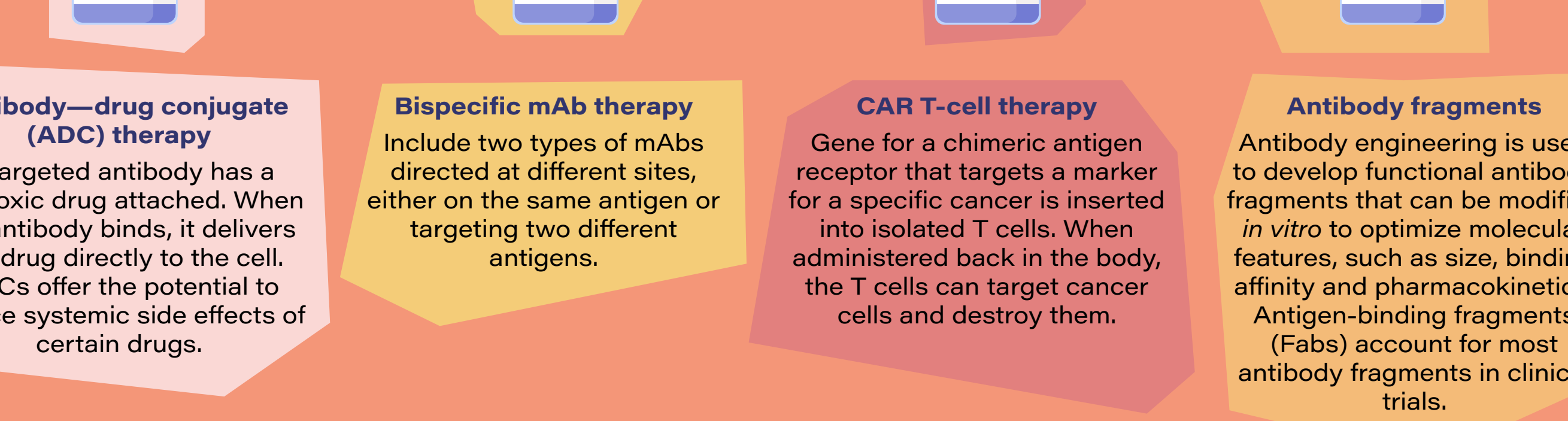
- Protein structure
- Post-translational modifications
- Function at the biomolecular and cellular levels

require mAb characterization. Some key analytical techniques adopted here include, but are not limited to:

- **Chromatography**
- **Liquid chromatography-mass spectrometry (LC-MS)**
- **High-performance liquid chromatography (HPLC) coupled with MS**

CLINICAL APPLICATION OF THERAPEUTIC ANTIBODIES PRESENT AND FUTURE

A number of different antibody therapeutics currently exist, including:



Antibody—drug conjugate (ADC) therapy
A targeted antibody has a cytotoxic drug attached. When the antibody binds, it delivers the drug directly to the cell. ADCs offer the potential to reduce systemic side effects of certain drugs.

Bispecific mAb therapy
Include two types of mAbs directed at different sites, either on the same antigen or targeting two different antigens.

CAR T-cell therapy
Gene for a chimeric antigen receptor that targets a marker for a specific cancer is inserted into isolated T cells. When administered back in the body, the T cells can target cancer cells and destroy them.

Antibody fragments
Antibody engineering is used to develop functional antibody fragments that can be modified *in vitro* to optimize molecular features, such as size, binding affinity and pharmacokinetics. Antigen-binding fragments (Fabs) account for most antibody fragments in clinical trials.

NOVEL THERAPEUTIC MODALITIES BEING EXPLORED...

Nanobodies

Over recent years, nanobodies – domains found on the heavy-chain antibodies of camelids – have garnered increased research attention. They:

- Possess high affinity
- Are highly stable
- Are efficient to produce
- Have low immunogenicity

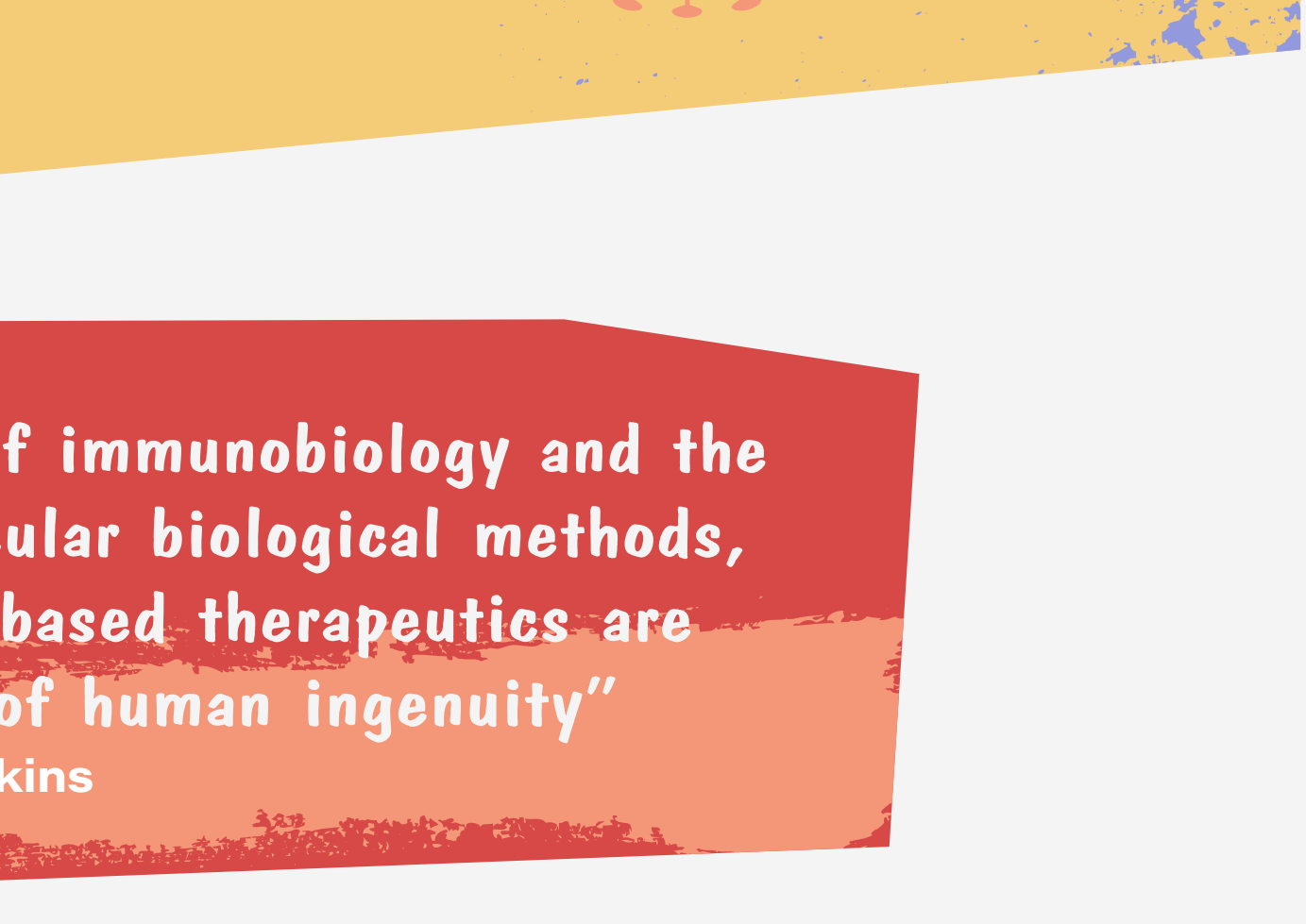
Existing mAbs can be limited due to their large size and – sometimes – low stability. Nanobodies may offer a novel antibody-based approach for treating solid tumors and other indications, such as coronavirus infections.

Broadly neutralizing antibodies (bNAbs)

bNAbs target a conserved region of the human immunodeficiency virus (HIV) viral envelope.

bNAbs have been shown to recognize and block entry of different HIV strains, in addition to recruiting immune cells to destroy already-infected HIV cells.

They therefore could be utilized as a preventive *and* therapeutic for HIV.



> "With increased understanding of immunobiology and the continued development of molecular biological methods, the possibilities for antibody-based therapeutics are bounded only by the scope of human ingenuity"
> **Goulet and Atkins**