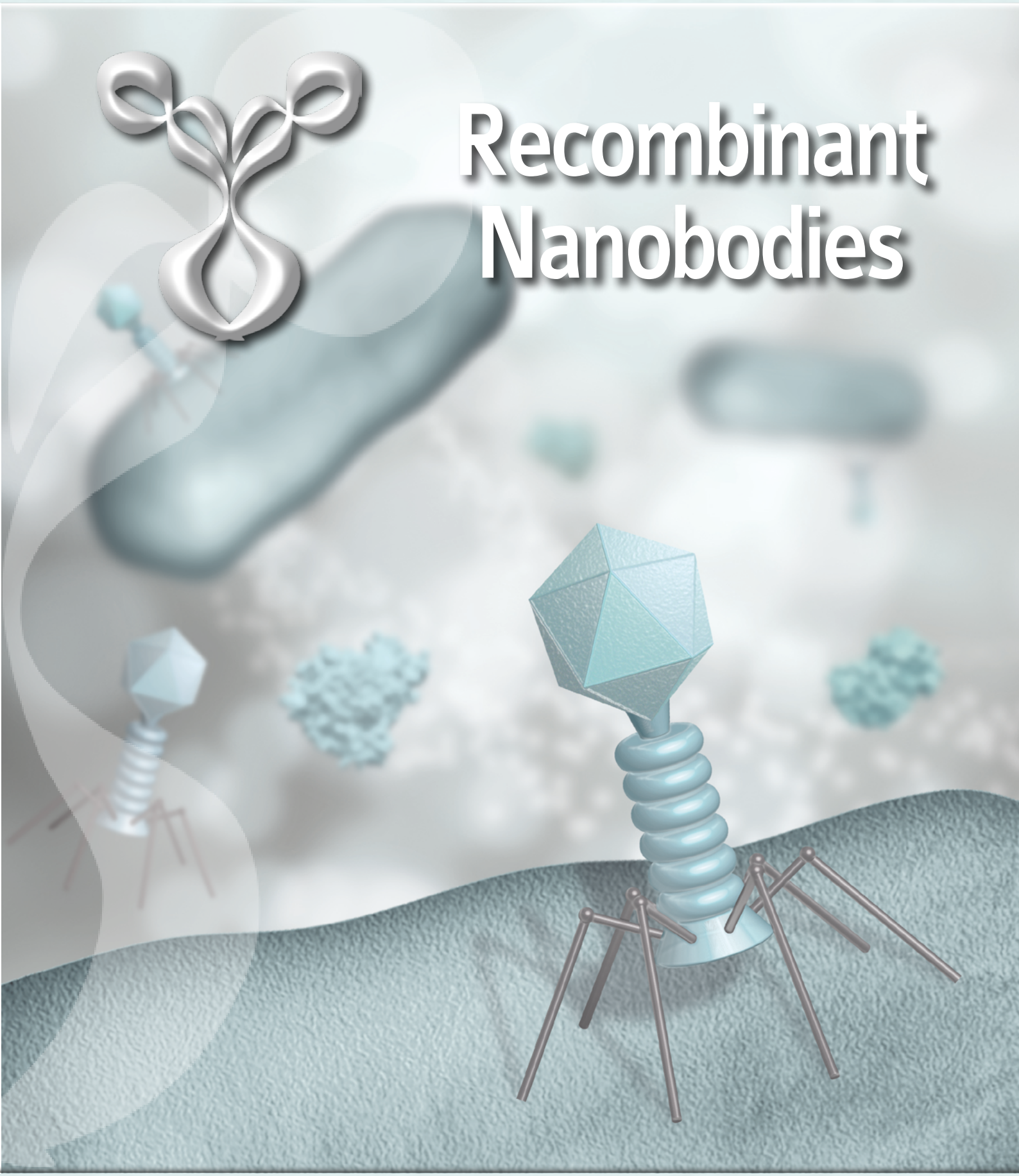
From Research to Discovery

covalab
R&D in Biotechnology

Antibody Development Services

Recombinant Nanobodies



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WHY USE NANOBODIES?

Human IgG
(150 kDa)

Fab fragment
(50 kDa)

ScFv fragment
(25 kDa)

Camelid IgG_2 / IgG_3
(80 kDa)

VHH fragment
(15 kDa)

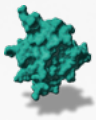
Immunoglobulins are very powerful tools which are used in many lab experiments. But over the last few years, their size has progressively become an issue for some applications. Many solutions have been engineered to overcome this problem, such as **Fab** or **single chain (ScFv)** fragments.

Camelids IgG_2 and IgG_3 possess only 2 heavy chains composed of two constant regions and a very versatile antigen binding domain called **VHH**. Using molecular engineering, high affinity VHH fragments of only 15 kDa called **Nanobodies**, which are more stable and soluble than conventional IgG, can now be produced in high scale, thus allowing to reach hardly accessible antigens and penetrate tissues easier.

WHICH TYPE OF IMMUNOGEN?

PROTEIN

To perform all required steps of the immunization protocol, which includes immunization, development of the screening method and immunoreactivity monitoring, we require a **few milligrams of the immunogen** *(recommended concentration: 2 mg/ml)*, which can be as:



Protein

In solution
Lyophilized
Within polyacrylamide gel

Microorganism

Inactivated virus
Inactivated bacteria
Inactivated yeast

Other

Cell or tissue extracts
Others *(please contact us)*

PEPTIDE — POST-TRANSLATIONAL MODIFICATION

Design

We use our in-house expertise to design **the most relevant peptides** to ensure the specificity of the nanobody.

We analyze **physicochemical properties** to predict immunogenicity and tertiary structure among others.

We ensure **minimal cross-reactivity** with non-relevant proteins by analyzing their homology with other sequences using alignment tools.

Synthesis

Peptides are synthesized using solid phase Fmoc chemistry, and analyzed by HPLC and mass spectrometry.

Length: 15 residues in average

Quantity: 20 to 25 mg

When developing an **anti-PTM** antibody, we synthesize one modified peptide as well as **one unmodified control peptide** to ensure the specificity of the resulting antibody for the modification.

Conjugation

Conjugation is usually carried out on a **cysteine residue** *(which can be added to the N- or C-terminus of the peptide if required)*, but other techniques can be used if necessary.

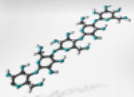
After synthesis, peptides are conjugated with one of the following carrier proteins:

KLH
Keyhole limpet hemocyanin

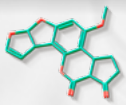
OVA
Ovalbumin

BSA
Bovine serum albumin

HAPTENS



Carbohydrates



Chemicals & Toxins

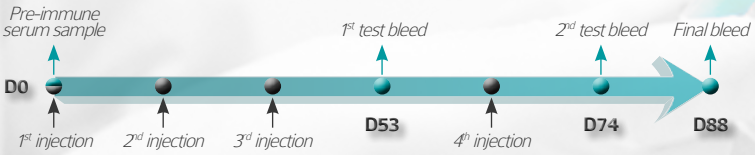
Natural or modified nucleotides

DETAILED PROTOCOL

IMMUNIZATION

I The first step of the procedure consists in injecting the immunogen into the host llama to generate an immune response, according to our 88-day exclusive protocol dedicated to this animal. Thanks to our 20-year experience and know-how, the number and frequency of injections are optimized real-time to ensure the best immunoreactivity / quantity of immunogen ratio.

Standard 88-day protocol



Each test bleed is assayed according to the detection method developed for the project. Our immunization protocols are adjustable according to the immunoreactivity of the sera. In case of weak immune response, additional injections and/or bleeds can be requested.

All experiments are undertaken by experienced and authorized staff following Health and Safety procedures, established according to the French legislation governing the use of animals in experiments.

You receive:

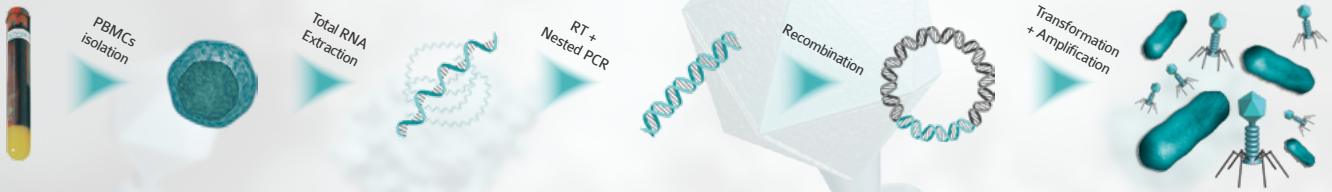
- Test bleeds so that you can run tests in your specific conditions in order to adapt the immunization protocol if the immunoreactivity is not as high as expected.

Our guarantee:

- The immunized animal is kept in the animal facility to perform additional total RNA extraction if necessary.

PHAGE LIBRARY CONSTRUCTION

II After confirmation of the secretion of antibodies into the bloodstream according to both our ELISA tests and your results, we proceed to the extraction of total RNA from PBMCs. Using suitable primers, retro-transcription is performed to obtain a library of cDNAs which are then inserted into phage vectors. Transformation into bacteria allows the expression of corresponding proteins at the surface of the phages.



Peripheral blood mononuclear cells (PBMCs) are isolated from the final bleed and total RNA is extracted.

Specific primers are designed to perform retro-transcription within the region of the genome coding for VHH and second nested PCR allows cDNA amplification.

cDNAs are integrated into our phage vector which is then used to transform competent bacteria for phage production.

Phages are produced by the bacteria and released into the culture medium upon lysis.

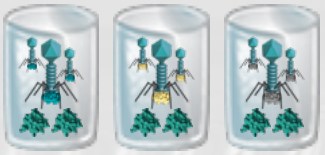
PANNING

III

Several rounds of selection are necessary to isolate the phages which carry the cDNA coding for the VHH of interest. By immobilizing the antigen in microtiter plate wells, only phages which can bind the antigen are retained while the others are removed. Transduction of bacteria using those phages allows enrichment and amplification of specific VHH-coding cDNAs.

The antigen is immobilized in the bottom of microtiter plate wells.

Loading



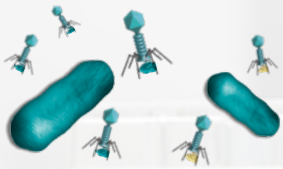
Phage preparations are loaded into the wells to allow them to bind to the antigen through the VHH expressed at their surface.

Washing

After washing out of unbound phages, remaining phages are tranduced into competent bacteria for enrichment and amplification.

Transduction & Amplification

Lysis & Purification

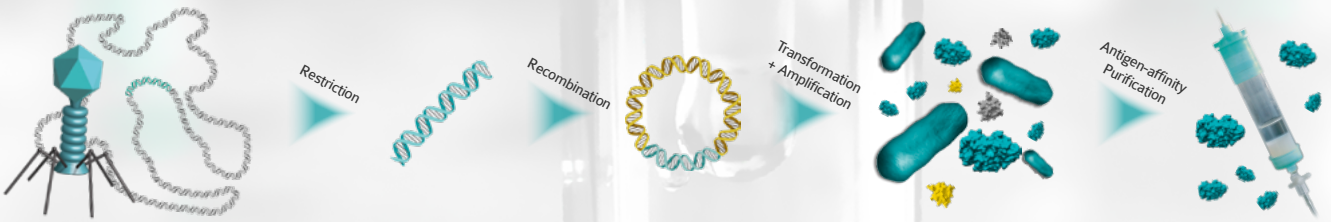


At the end of each panning stage, cDNAs coding for VHH specific to the antigen are isolated.

HIGH SCALE PRODUCTION

IV

The cDNAs coding for VHH of interest can then be used for high-scale production. The nucleic acid sequences are extracted from phage genome and are subcloned into our expression vector. Nanobodies are then produced after transformation and growth of competent bacteria followed by affinity chromatography purification.



Using restriction methods, cDNAs are extracted from the genome of the phage of interest.

Inserts are integrated into our high yield expression vector, which is then used to transform competent bacteria.

Specific nanobodies are purified from culture supernatants by affinity chromatography.

You receive:

- VHH preparations purified from bacteria culture medium by antigen-affinity chromatography.

Our guarantee:

- All cDNAs coding for specific VHH are kept frozen at -80°C in our laboratory.

PROTOCOL OVERVIEW

I

Immunization

4 injections & 2 test bleeds
88-day standard protocol

Monitoring

ELISA assay on each test bleed
Comparison of sera immunoreactivity over time

IMMUNIZATION

STOP

At the end of this step, polyclonal antibodies are already available. Upon request, large serum samples can be collected and affinity purified to obtain highly specific heavy chain IgG.

II

Extraction

PBMCs isolation
Total RNA extraction and retro-transcription

Phage production

Phage vector recombination
Bacteria transformation, growth and lysis

PHAGE LIBRARY CONSTRUCTION

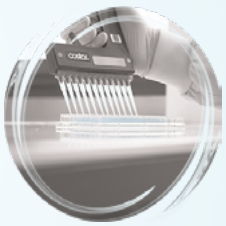
III

Selection

Selection of phages of interest based on antibody-antigen interaction
Elimination of unbound phages

Amplification

Amplification of phages of interest by transduction of bacteria
Purification of phages preparations



PANNING

STOP

At the end of this step, cDNAs coding for VHH fragments specific to the antigen are identified. Upon request, we can provide you with purified specific cDNAs included in the phage genome so that you can perform subcloning into your own expression vector.

IV

Recombination

Extraction of cDNA from phages of interest
Recombination in high yield expression vector

Production

Transformation into competent bacteria and high scale culture
Lysis and affinity chromatography purification

PRODUCTION

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R&D in Biotechnology

Services

From project design to antibody production

Custom projects

Protocols adapted to your requirements

Technical support

Advice by experts in immunology & molecular biology

Ethics

Approved Health & Safety procedures

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