



Work Package 4: Binders

Introduction

Most of the current immunoassay-based methods developed aim to detect a single type of target molecule from a sample. The aim of BioCop is to design assays that are able to screen multiply classes of antigens in one test.

Broad specificity and high-quality binding molecules are needed for this success, and state-of-the-art technologies are utilised for generating these binders. The objective of Work Package 4 is to generate and deliver binder molecules for many chemical contaminants, which will then be used by other work packages in BioCop.

Work Package Progress and Results

Antibodies

The generation of polyclonal antibodies have been used to bind:

(i) Several biomarkers of hormone abuse in cattle (WP2) such as myostatin, IGF-1, inhibin, propeptide of type III procollagen, α 1-antitrypsin, α 2-antiplasmin, endopin-1, IGFBP-2, L-lactate dehydrogenase B-chain, serotransferrin

(ii) At least 8 targeted fluoroquinolones (WP8)

(iii) Paralytic shellfish toxins (WP5)

Recombinant antibodies

In BioCop, promising monoclonal antibodies have been engineered by directed evolution. Rationally designed antigen derivatives have been used for selecting improved binders.

Anti-fluoroquinolone Fab selections have been engineered by mutagenesis and new binding variants have been selected with phage display technology. This has resulted in a binder that can detect 7 out of 8 targeted fluoroquinolones.

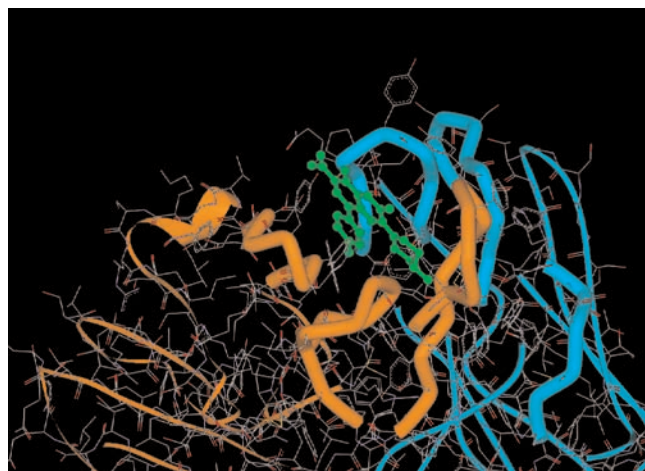


Figure 1. An example of small sized derivative synthesized in WP2 for antibody selections from recombinant antibody library. These derivatives presumably evade the common bridge effect which is found when animals are immunised with protein-coupled antigens.

From the anti-PSP Fab mutant library, mutants with improved binding against the problematic GTX1/4 were selected. Purified mutants were further delivered for evaluation.

The universal recombinant antibody library intended for development of binders for small molecular antigens has been constructed. This synthetic antibody library is based on state-of-the-art knowledge of immunoglobulin (antibody) genes, 3D structure and bioinformatics.

The selection of strobilurin specific antibodies with immunisations or recombinant techniques was unsuccessful despite using different methods.

Recombinant proteins

In addition to conventional antibodies, natural targets of toxins or pesticides can also be used as specific binders. The genes-to-proteins approach with fusions to solubilising partners was used to identify the binders for saxiphilin and strobilurin.

Cloning and expression of these targeted receptors was unsuccessful.





Since biomarkers of hormonal abuse are not available commercially, they are cloned and produced: a total of seven proteins (endopin, α 1-antitrypsin, α 2-antiplasmin, IGFBP, inhibin 1-32, serotransferrin and L-lactate dehydrogenase) were delivered in mg quantities for polyclonal antibody production.

Artificial receptors

This approach of design was used to obtain a sensor for organochlorine pesticides based on a suitable receptor molecule.

Two molecules have been identified as being able to bind organochlorine pesticides (OCP). These molecules (4-sulfonic-calix[6]arene [A] and 4-sulfonic-calix[8]arene [B]) form complexes with pesticides by inclusion in their hydrophobic cavity. (Figure 2)

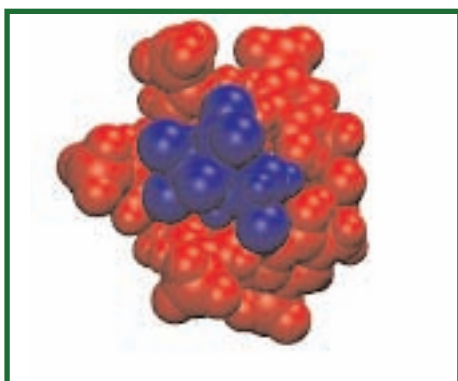


Figure 2. Artificial receptor approach – 3-dimensional structure of a complex between a calixarene A receptor and the heptachlor pesticide as obtained from molecular dynamics stimulations

Binding to four organochlorine pesticides; endosulfan, heptachlor, lindane and chlordan has been obtained.

Benefits of the BioCop Project

Consumer

Consumers are becoming increasingly concerned with chemical contaminants in food, and the need to know about food-related risks.

Since the food and feed industries are responsible for the safety of their products; by increasing the monitoring of such residues, consumers will have more trust in the food that is being consumed.

Food Industry

Food businesses need more and more rapid and inexpensive test methods to be able to detect the absence of contaminants in their produced food. Therefore the necessity to have performing binders to develop these types of methods is essential.

Scientist

A number of papers referring to antibodies produced and characterised within WP4 have been published. These include the following publications, *Analytica Chimica Acta*, 2008 (WP8), *Food Additives and Contaminants* 2009 (WP8), *Analytica Chimica Acta*, 2009 (WP2).

Training/Workshops

A Hands-on course on the phage display technique was recently held in Turku (Finland). During the course, the participants performed one panning cycle using in-house phage antibody library and biotinylated antigens.

Future Activity

Additional immunisation schedules are planned for future activity.

Frequently Asked Questions

Question:



How does a classical immunisation for polyclonal antibodies production occur?

Answer:



All polyclonal antibodies are raised in rabbits by subcutaneous injection, with 0.2mg immunogen emulsified with Freund's complete/incomplete adjuvant (first injection/all injections following this) Injections are administered on a fortnightly basis and then, from the third injection, they are administered at the rhythm of one injection every 28 days. Test bleeds are later collected 10 days after each immunisation (from the third immunisation onwards).

