MACAFFIN : Increasing humanized antibody affinity

Introduction

Monoclonal antibodies are playing an increasing role in drug therapy. Most of them are generated as rodent antibodies and induce an adverse immune response when injected into humans. Modifying antibody sequences aims to decrease their immunogenicity but often leads to a loss in either affinity or specificity or both. This project aims to develop a quick humanisation process of murine antibodies leading to the production of high affinity/specificity humanised antibodies. The developed procedure includes four steps :

1. **Indentification of the murine CDR sequences and cdr-grafting**

   Antibody humanisation by CDR-grafting consists of the insertion of the murine complementary determining regions (CDR) into a human variable regions framework. Antibody specificity and affinity are determined by CDR sequences but also CDR structures and relative positions. So that human framework sequences and murin CDR séquences have to be chosen carefully by bioinformatics analysis (figure 1).

2. **Molecular modeling and site-directed mutagenesis**

   If the grafted antibody shows a loss in affinity or specificity, amino acids affecting CDR positioning and light and heavy chain angulation are selected for mutation back to the murine sequence.

3. **Production of humanised antibodies**

   Engineered antibodies are produced in human HEK293 cell lines and affinity purified.

4. **Specificity and affinity détermination**
   - ELISA
   - KD, Koff, Kon are measured by bio-layer interferometry.

We are looking for partnerships to submit new projects within the fields of antibody engineering or protein engineering.

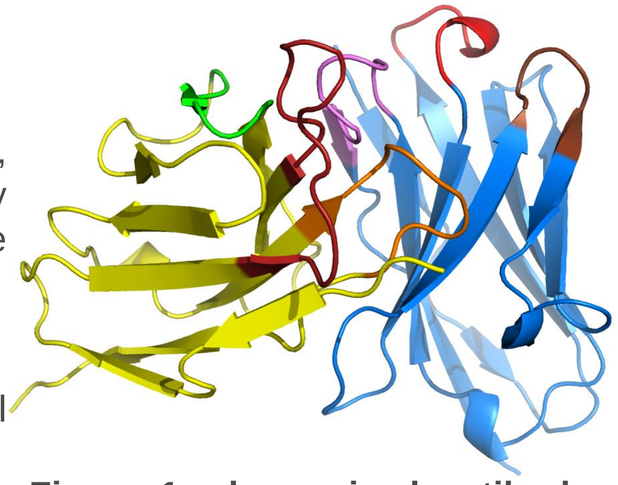

Figure 1 : humanized antibody : variable region modeling. Mouse derived CDR, Chothia definition (H1 red, H2 brown, H3 violet, L1 firebrick, L2 green, L3 orange), human heavy chain framework (blue), human light chain framework (yellow).

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