T-ECO

iGEM Team Tuebingen 2014

Our Project

The purpose of our project is to create an implementation to convert blood groups of type A, B and AB of the ABO blood group system into blood of the type 0 or Bombay type. Blood type 0 is the universal transfusion blood type, which can be received by every person. An exception is the rare Bombay type (Oh).

The main goal of our project is the covalent immobilization of three conversion enzymes onto a matrix using different tags. Furthermore the activity of the immobilized enzymes will be tested.

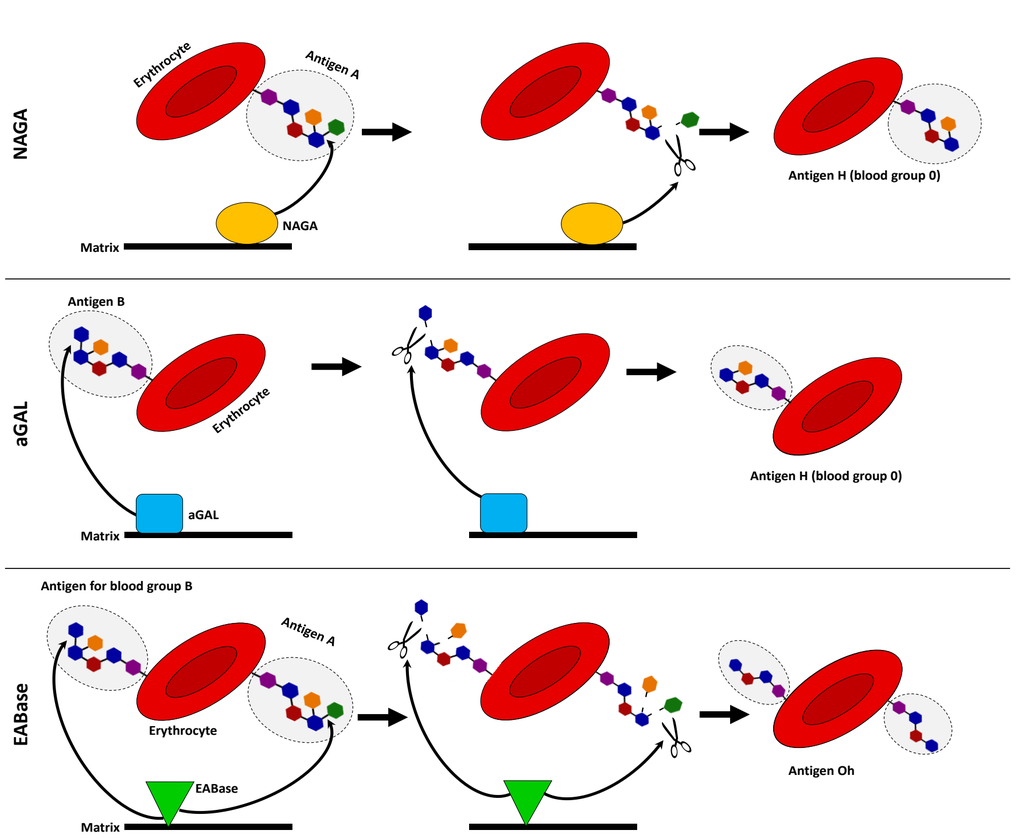

Figure 1: Graphical abstract of the T-ECO conversion system

Blood conversion enzymes

Three different enzymes are used in our project to achieve conversion of blood types A, B and AB. These enzymes are:

- **N-Acetyl-Galactosaminidase (NAGA)**
  from *Elisabethkingia meningosepticum* (blood type A to 0 and AB to B)
- **α-Galactosidase (aGAL)**
  from *Bacteroides fragilis* (blood type B to 0 and AB to A)
- **Endo-β-galactosidase (EABase)**
  from *Clostridium perfringens* (blood type A, B and AB to Oh)

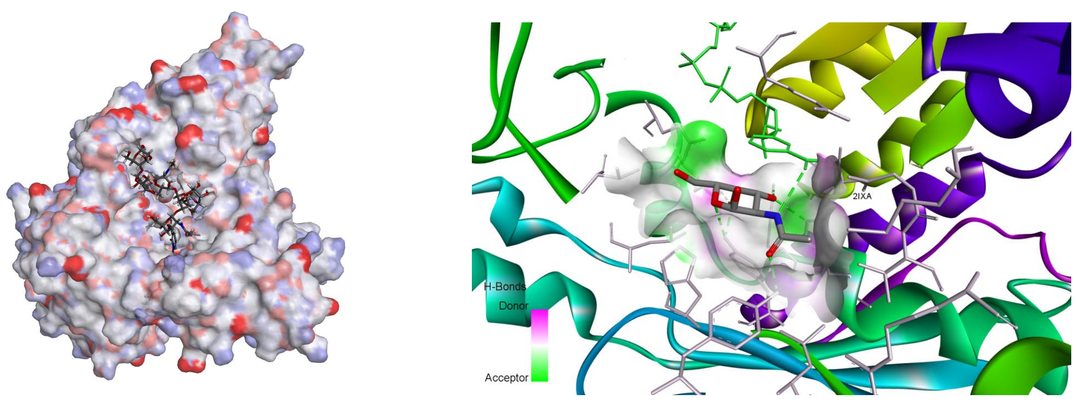

Figure 2: Active site of the NAGA bond to N-acetylgalactosamine and NAD^+

Cloning methods

For cloning purposes two different *E. coli* strains are used:

- NEB5α – cloning strain for plasmid amplification
- BL21(DE3) – expression strain for tag-coupled enzymes

Blood group system ABO

The ABO antigens consist of glycosylated proteins on the erythrocyte's surface. Individuals with blood types A or B both have a distinct glycosylation that serves as an antigen. Individuals with blood type AB have both antigens.

Blood group 0 antigen covers the primitive glycosylation of five sugars, the antigens of blood type A and B therefore cause no immunoreaction. The Bombay type blood (Oh) is characterized by even less sugar residues. Therefore, people with Bombay type blood can only accept Bombay type donated blood.

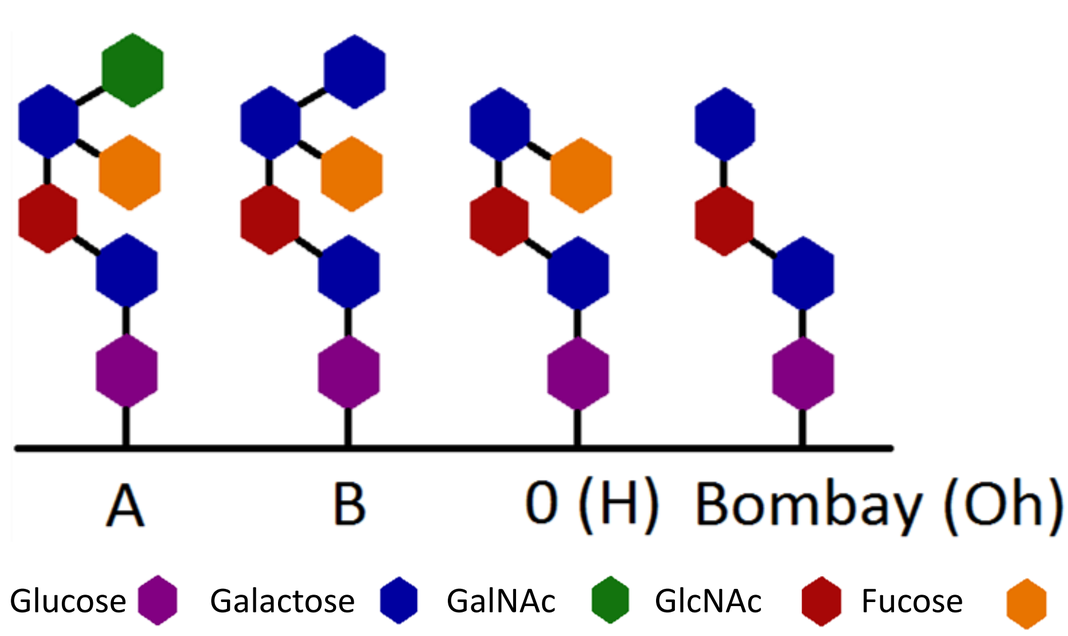

Figure 3: Overview of the glycosylation of the erythrocytes

Immobilization methods

For the immobilization on the resin three different tags are used:

- **SNAP-tag**:

  The enzyme is coupled with the SNAP-tag and expressed together as one protein chain. The SNAP-substrate is then fused to the resin.

- **SpyTag**:

  The SpyTag-system consists of two parts, the SpyCatcher (fixed to the enzyme) and the SpyTag (synthetic peptide attached to a membrane). Both parts are fused together, yielding an immobilized enzyme.

- **Ssp GyrB split intein**:

  The intein-tag is derived from a self-splicing protein. When both parts approach each other, the intein parts fuse, splice themselves and leave behind an immobilized enzyme on the resin.

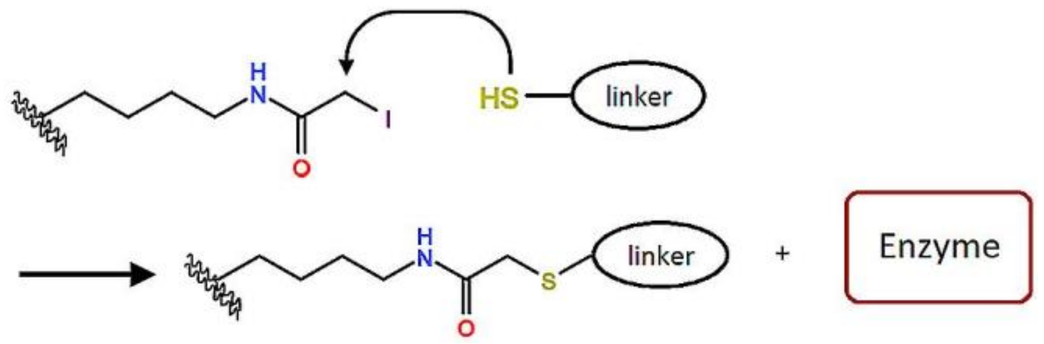

Figure 4: Formation of thioether bond of terminal cystein residue and activated resin used for SpyTag and Intein

Methods of analysis

The activity of the soluble enzymes is determined in a simple clotting test with blood antibodies, commonly used to identify a patient's blood group.

The immobilized enzymes' activity is measured with FACS using special fluorescein coupled antibodies.