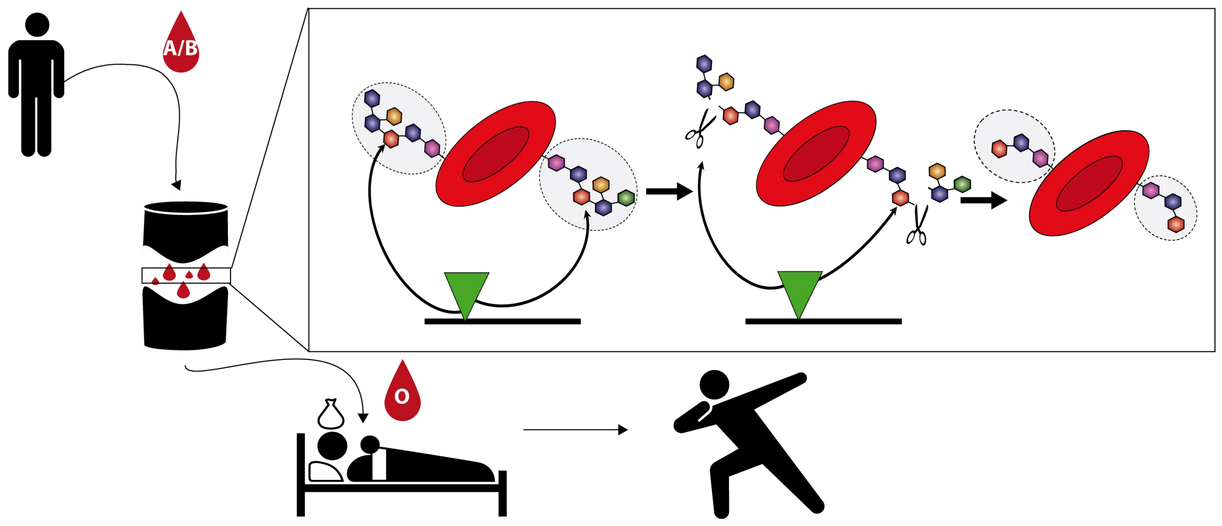
T-ECO

iGEM Team Tuebingen 2014

Team Tuebingen

Our Project

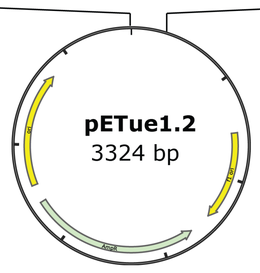
The purpose of our project is to create an implementation to convert blood groups of type A, B and AB of the ABO blood group system into blood of the type 0 or Bombay compatible. Blood type 0 is the universal transfusion blood type.



The main goal of our project is the expression and covalent immobilization of three conversion enzymes onto a matrix using different tags.

Cloning

Introduced features of our designed expression plasmid pETue1.2



Enzyme	Tag	Plasmid
NAGA	SNAP-tag	pSB1C3
aGAL	SpyTag	pETue1.2
EABase	Split intein	pQE-82
		pSBX1K3

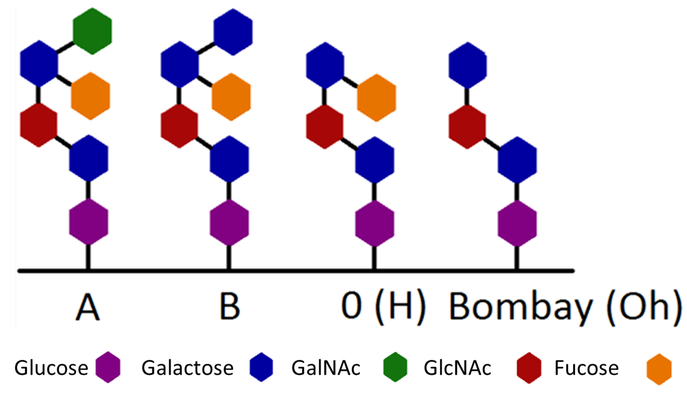
We cloned the enzymes mentioned above with all tags in different plasmids. All in all we cloned 36 different constructs. We submitted 4 BioBricks, among them is one intein BioBrick and the 3 conversion enzymes.

Tuebingen Erythrocyte Converter to O T-ECO

Blood group system ABO

The ABO antigens consist of glycosylated proteins on the erythrocytes surface. Blood group 0 antigen covers the primitive glycosylation of five sugars. Blood types A or B have additional terminal sugars. Individuals with blood type AB have both antigens.

The Bombay type erythrocytes (Oh) are characterized by less sugar residues. Therefore, people with Bombay type blood can only accept Bombay type donated blood.



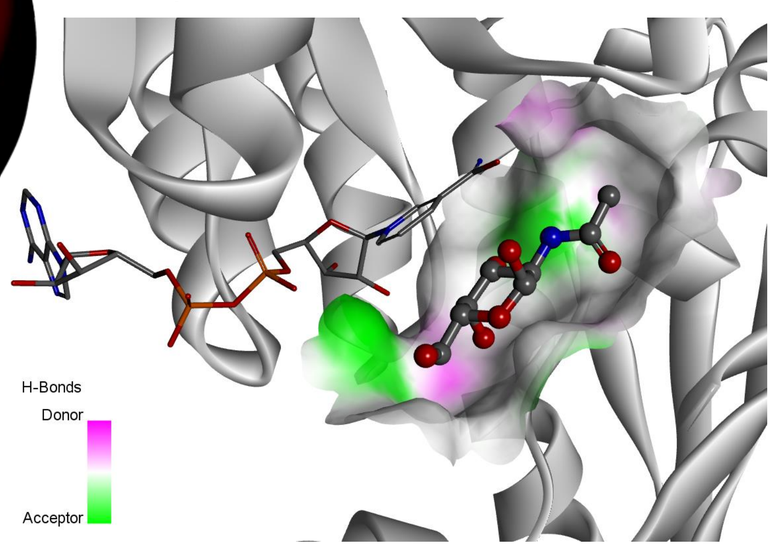
Enzymes

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

α-N-Acetylgalactosaminidase (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)

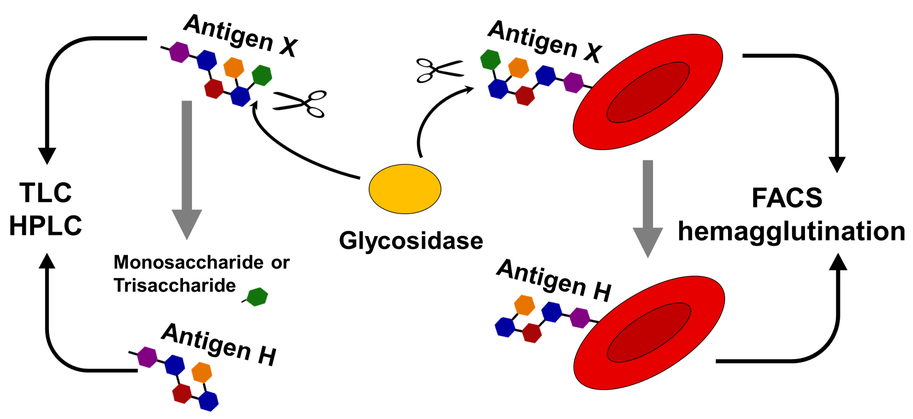
Endo-β-galactosidase (EABase)
from *Clostridium perfringens* (blood type A, B and AB to Oh compatible blood)



Binding pocket of NAGA (2IXB) with GalNAc and NAD^+ in close proximity

Validation

The conversion of red blood cells with immobilized enzymes is challenging as both the enzyme and the substrate are bound to a large particle.

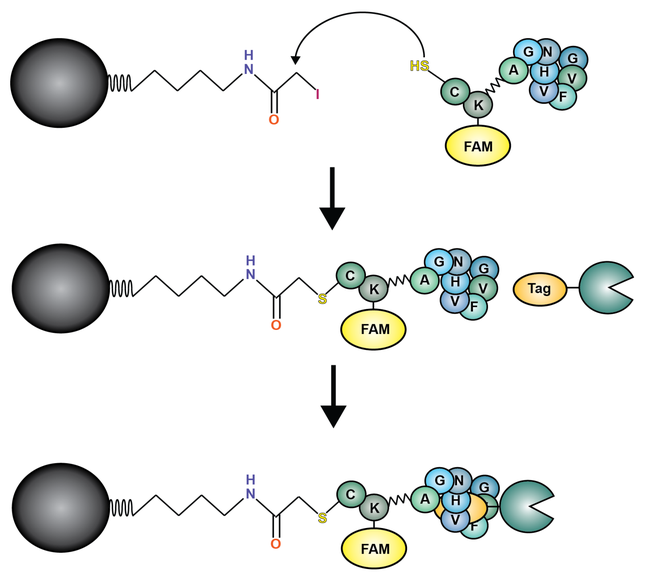


We developed a strategy to monitor the activity of our enzymes at every stage using a soluble oligosaccharide as substrate and thin-layer chromatography or high-performance liquid chromatography as read out. The presence of blood antigens on the erythrocyte surface will be detected by hemagglutination and fluorescence activated cell sorting.

Immobilization

For the immobilization on a resin the enzymes are expressed as fusion proteins with three different tags:
SNAP-tag, SpyTag, Ssp GyrB split intein

SpyTag and Intein can be used with synthetic peptides. The SNAP-tag reacts with O^6-benzylguanine (BG). First the peptides/BG need to be bound to a matrix. In a second step they interact specifically with their corresponding tag, thereby forming a covalent bond linking the protein of interest to the matrix.



Public Awareness

We informed Tuebingen's population about genetic engineering by participating in an event coordinated by all German iGEM teams, the SynBioDay.
Furthermore we invited a local school class to our institute where we discussed the hallmarks of synthetic biology and they got the opportunity to practice basic lab techniques.

Collaborations

We verified team Heidelbergs protein samples for protein-cyclization and protein-protein-coupling by mass spectrometry. Team Heidelberg kindly sent us samples of their expression plasmids pSBX1K3 (BBa_K1362093) and pSBX4K5 (BBa_K1362097).

eurofins
greiner bio-one
ROTH
GenScript Make Research Easy
NEW ENGLAND BioLabs Inc.
immatics
GATC BIOTECH
ELICITYL Glycoproducts for life sciences
Biozym SCIENCE IS OUR BUSINESS
PURIMEX DNA / RNA Oligonucleotides
SERVA Electrophoresis
QIAGEN
SnapGene Software for molecular biology
Thermo SCIENTIFIC