Contract work case study
Expression and purification of mutated dengue Envelope proteins on behalf of Excivion

Background:

Dengue is a mosquito-borne disease caused by one of the four dengue virus (DENV) serotypes 1-4, with over 96 million cases annually. People who suffered a primary dengue infection in the past are at a higher risk of developing dengue haemorrhagic fever and dengue shock syndrome during secondary infection due to antibody-dependent enhancement (ADE). In short, antibodies generated against the first serotype during primary infection are fully protective against re-infection with the same serotype. However, these same antibodies will bind to, but not neutralise, a different serotype during secondary infection. In addition to its regular route of infection, virus opsonised in this way can then enter cells via Fc receptors, providing an additional cell entry mechanism which exacerbates viraemia and symptoms of infection.

The DENV envelope is composed of the Envelope protein (the major glycoprotein) and the Membrane protein (the minor glycoprotein). The fusion loop is a region of the Envelope protein that is responsible for fusion of the virion and cell membranes and is necessary for infectivity. It is well conserved among Envelope proteins from different serotypes, and among flaviviruses in general. This region is known to contribute significantly to the effects of ADE. Therefore, Excivion decided to produce a candidate recombinant Envelope vaccine in which the fusion loop is made inaccessible to the immune system by introduction of one or more glycosylation sites within the fusion loop. The Native Antigen Company was approached by Excivion to produce four antigens for pre-clinical studies.

Project details:

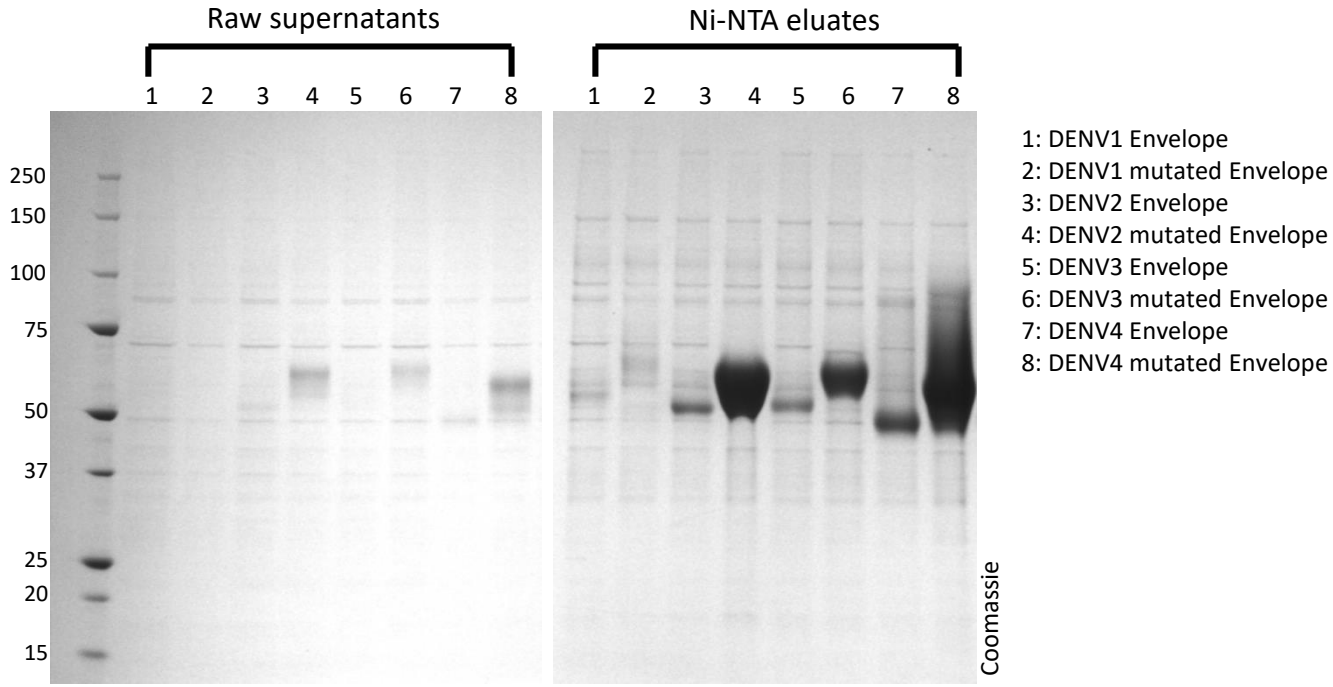
All antigens were produced by transient transfection of human embryonic kidney 293 (HEK293) cells, followed by purification of secreted protein from culture supernatant. The C-terminal transmembrane domain of each of the Envelope proteins was replaced by a glycine-serine linker and a 6x histidine-tag (His-tag), and the modified open reading frames were synthesized "de novo". The synthetic constructs were transferred to in-house expression vectors.

To check if the proteins were secreted and could be effectively purified a feasibility study was performed as follows:-

HEK293 cells were transiently transfected with the resulting four constructs. Culture supernatants were then harvested and concentrated using spin concentrators. The retentate from spin concentrators was then applied to Ni-NTA immobilised metal affinity chromatography (IMAC) spin columns alongside wild-type Envelope protein as controls. Eluates were analysed by SDS-PAGE (Fig 1a). Constructs for DENV2, DENV3 and DENV4 HG-Env proteins were well expressed and could be purified by IMAC. However, the yield of DENV1 HG-Env was low, but was consistent with yields seen for WT DENV1 Envelope protein.

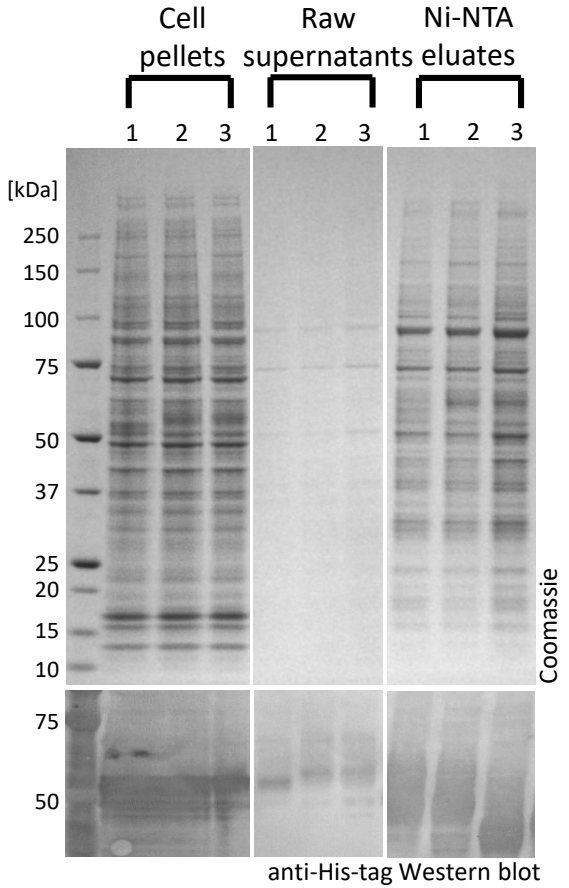
Large-scale expression and purification was undertaken with culture volumes ranging between 0.5L and 20L, dependent on yields seen in the feasibility study. Volumes up to 1L were handled directly, larger volumes were concentrated using tangential flow filtration (TFF). IMAC was performed using AKTA purification systems, and collected fractions were analysed on SDS-PAGE (Fig. 2a). The purity of most antigens was sufficient for the customer's downstream needs so that no further purification steps were needed. Ion exchange chromatography was performed for DENV1 HG-Env to increase antigen purity to >95%. A buffer exchange to physiological conditions was performed, followed by determination of protein concentration by bicinchoninic acid (BCA) assay. A final SDS-PAGE assessment confirmed specified targets for purity (>95%) were met (Fig. 2b).

Fig. 1a



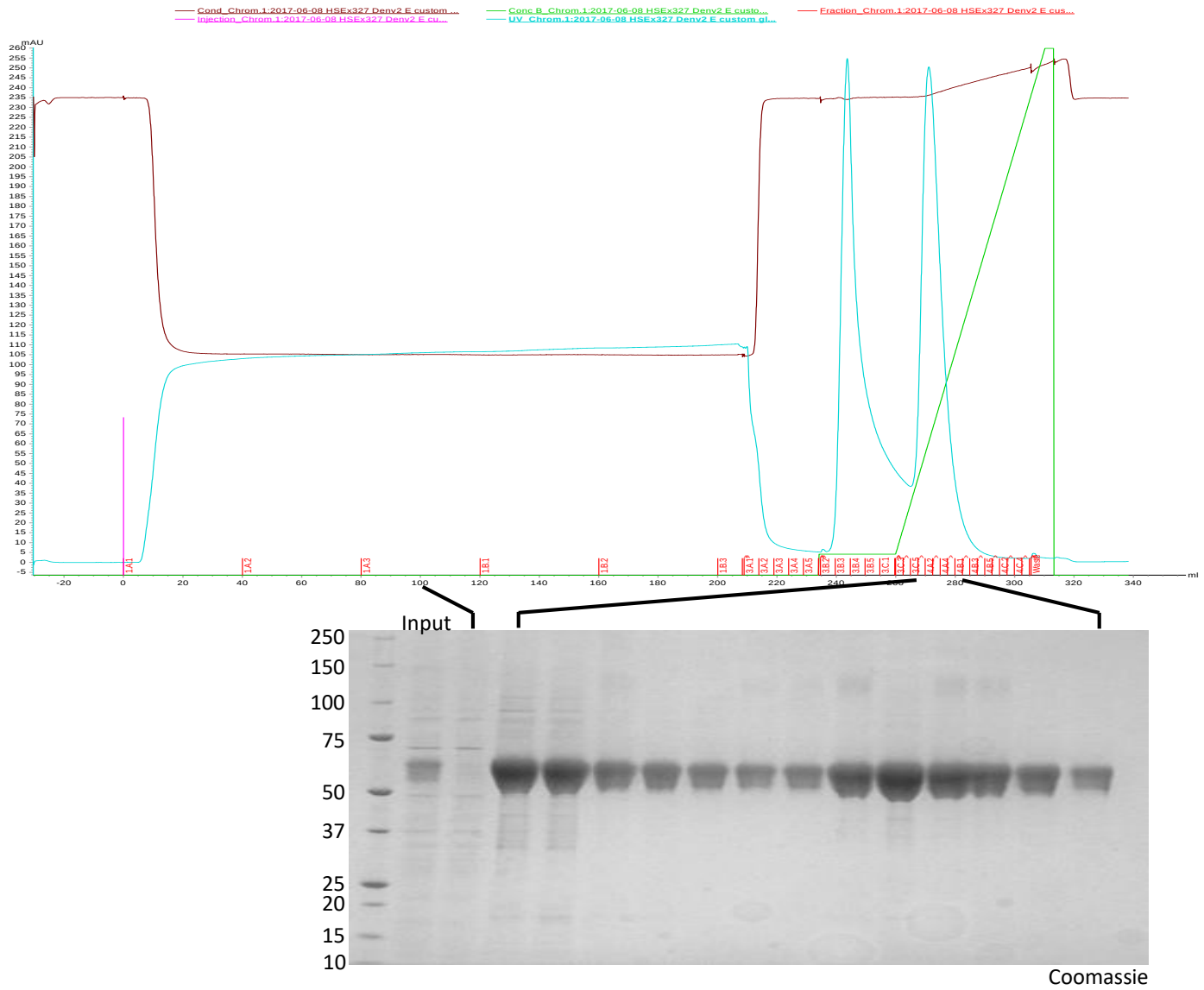
1: DENV1 Envelope
2: DENV1 mutated Envelope
3: DENV2 Envelope
4: DENV2 mutated Envelope
5: DENV3 Envelope
6: DENV3 mutated Envelope
7: DENV4 Envelope
8: DENV4 mutated Envelope

Fig. 1b



1: DENV1 Envelope
2: DENV1 mutated Envelope, variant 1
3: DENV1 mutated Envelope, variant 2

Fig. 2a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37
25
20
15
10
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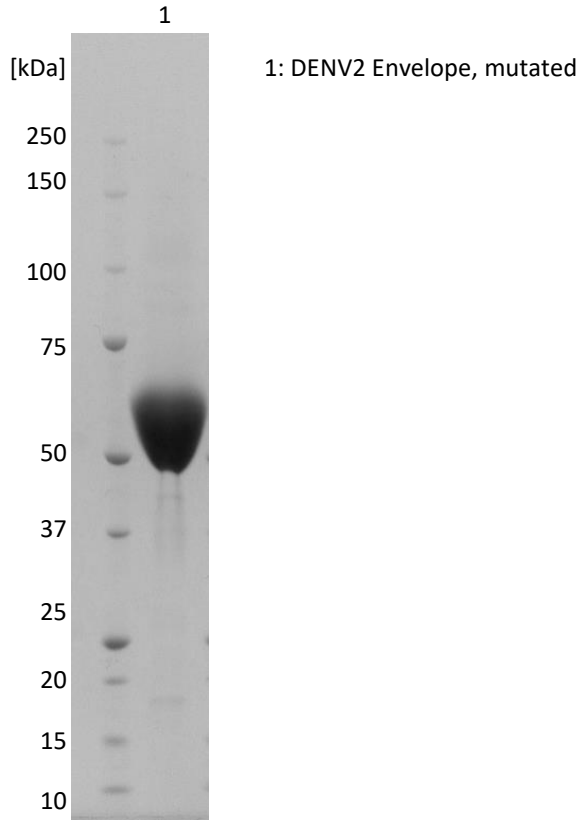
Fig. 2b



1: DENV2 Envelope, mutated