Expression and purification of Crimean Congo Haemorrhagic Fever virus GN and GC glycoproteins in insect cells

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INTRODUCTION

Crimean-Congo Haemorrhagic Fever (CCHF) is a serious virus (v) disease primarily transmitted to humans by ticks or infected blood/tissue. The disease is endemic in Africa, the Balkans, the Middle East and Asia. The mortality rates are up to 40% and there is no licenced vaccine available.

Vaccine generation attempts to date have been unsuccessful due to safety (inactivated virus particles) and efficiency (DNA-based vaccines) problems.

An alternative approach is to generate a sub-unit CCHFv vaccine based on the viral immunogenic glycoproteins, GC and GN (Fig 1A). Baculovirus insect cell expression system offers a safe and cost-effective platform for the generation of such vaccines.

In an earlier phase of this project, CCHF GN and GC proteins were produced using the baculovirus expression system and their immunogenicity was shown in mice (Fig 1B).

The aim of the current project was to optimise the production and purification of CCHFv GC and GN proteins. This was carried out by generating several versions of the proteins including truncated forms (Fig 1Aii) and the use of different purification tags.

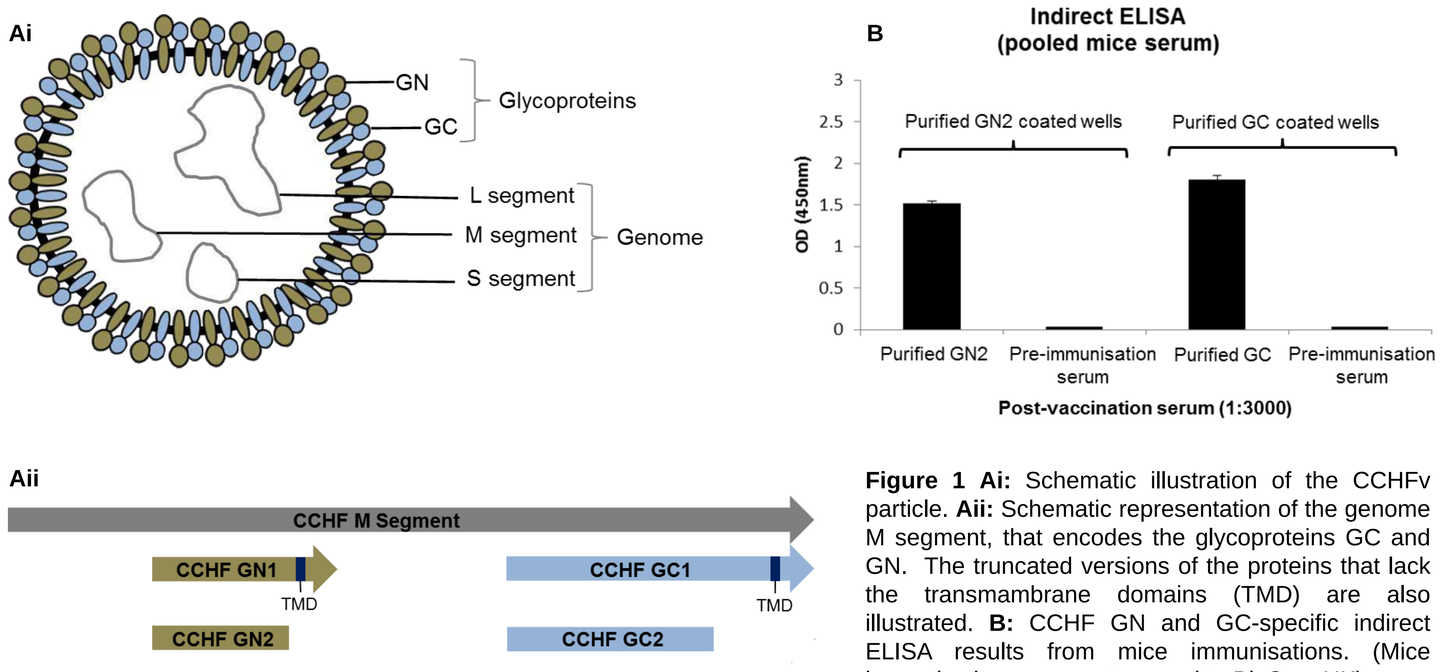


Figure 1 Ai: Schematic illustration of the CCHFv particle. **Aii:** Schematic representation of the genome M segment, that encodes the glycoproteins GC and GN. The truncated versions of the proteins that lack the transmambrane domains (TMD) are also illustrated. **B:** CCHF GN and GC-specific indirect ELISA results from mice immunisations. (Mice immunisations were outsourced to BioServ UK).

RESULTS

CCHFv GN1 and GC1 production from different virus strains

CCHFv has seven major geographical strains (Table 1) and their genome M segments have slight amino acid (aa) variations, which are believed not to affect the immunogenic epitopes of the glycoproteins. Therefore, we have investigated the effect of these aa differences on the production of the recombinant proteins in insect cells.

Initially 21 recombinant baculovirus constructs (V2v001-V2v021) were generated using the *flash*BAC™ system. Full-length GN of each strain was generated as a histidine-tagged protein and full-length GC of each strain was generated as both histidine and maltose binding protein (MBP)-tagged proteins in insect cells.

All proteins were successfully produced in Sf9 insect cells, however, the solubility of the proteins varied among different strains.

Strains A, B, C and G were taken forward and their solubility was tested in triplicate to examine reproducibility, which have confirmed strains A and G to be the optimum for protein production/solubility (Fig 2).

The results also shown that the use of different purification tags affected the protein solubility observed with the GC protein (Fig 2ii and iii).

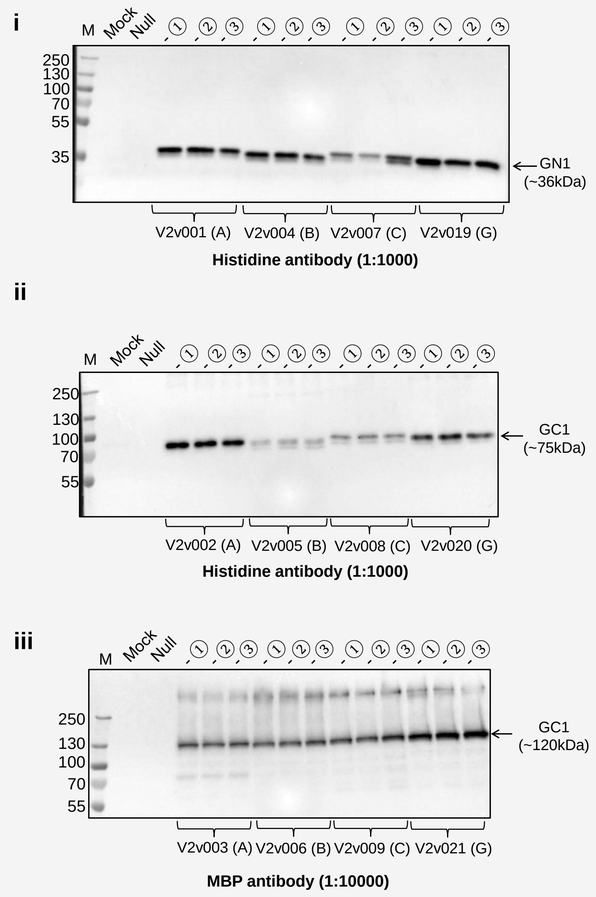
Table 1: CCHFv strains and corresponding project codes

Strain	Code
AF467768	**A**
Ard15786	**B**
UG3010	**C**
Oman	**D**
HULV-100	**E**
AP92	**F**
Ard39554	**G**

Figure 2: Solubility testing of CCHF GN and GC proteins in triplicate.
Sf9 cells (1x10^6 cells/dish; replicates 1-3) were infected with corresponding viruses using 5 multiplicity of infection (moi). The cells were harvested at 72 hours post infection (hpi) and resuspended in solubilisation buffer (1% NP40, 150mM NaCl and 50mM Hepes, pH8). Clarified supernatant fractions were analysed using SDS-PAGE followed by western blotting using target-specific antibodies.

i: Histidine-tagged CCHF GN1 proteins
ii: Histidine-tagged CCHF GC1 proteins
iii: MBP-tagged CCHF GC1 proteins

Figure 2



CCHFv GN and GC purification from insect cells

Based on strains A and G, several additional constructs were generated (V2v022-V2v065). These included both the full-length and the truncated versions of CCHFv GN and GC proteins with either MBP, histidine or strep affinity purification tags at carboxy or amino terminal ends of the proteins.

These constructs were screened by small-scale batch purification analysis. The use of strep tag was found to be the optimum due to the highest purity profile achieved compared to other purification tags tested (data not shown).

Five constructs (Fig 3A) were taken forward and purifications were carried out from either 1L Sf9 cell pellet for constructs; V2v031, V2v036, V2v039 and V2v055 or 1L culture medium for V2v045 construct, which was shown to be secreted (Fig 3B).

Truncating the GC protein was shown to increase the protein yields greatly (Fig 3A).

Possibility of dimer/trimer formation of the GN protein was observed, however, further analysis is required to confirm this.

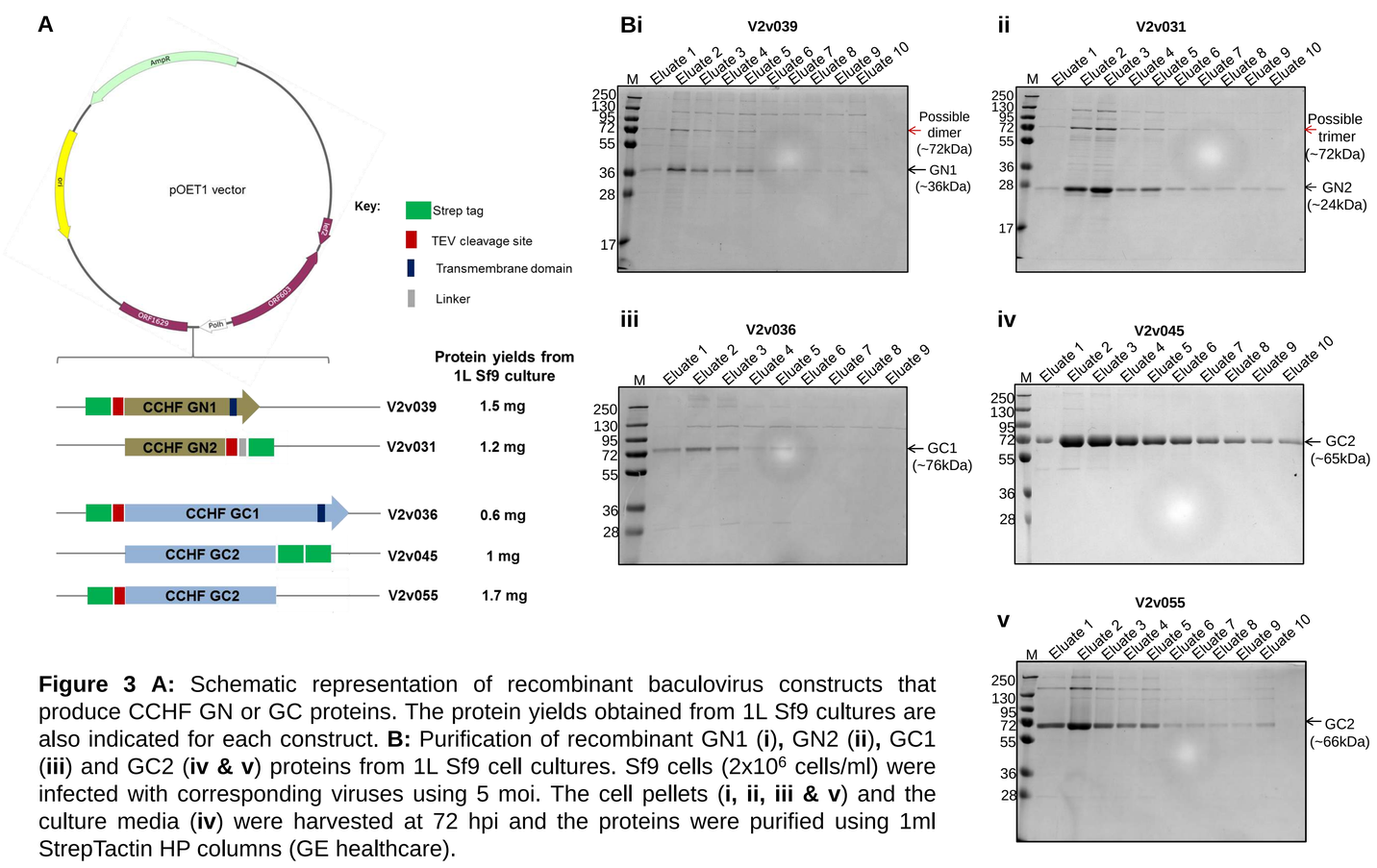


Figure 3 A: Schematic representation of recombinant baculovirus constructs that produce CCHF GN or GC proteins. The protein yields obtained from 1L Sf9 cultures are also indicated for each construct. **B:** Purification of recombinant GN1 (**i**), GN2 (**ii**), GC1 (**iii**) and GC2 (**iv & v**) proteins from 1L Sf9 cell cultures. Sf9 cells (2x10^6 cells/ml) were infected with corresponding viruses using 5 moi. The cell pellets (**i, ii, iii & v**) and the culture media (**iv**) were harvested at 72 hpi and the proteins were purified using 1ml StrepTactin HP columns (GE healthcare).

CONCLUSION

CCHFv GC and GN proteins and their variants can be produced to high yields using the *flash*BAC™ insect cell expression platform.

Purification of the recombinant CCHFv GC and GN proteins are best achieved using a strep tag.

The immunogenicity of the optimum constructs are currently being analysed in a mice vaccination study.

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