MOL231: Optimization, expression and preliminary NMR studies on the Honeybee Vitellogenin von Willebrand Factor Domain

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Background

Vitellogenin (Vg) is a large multidomain protein of approximately 180 kDa that belongs to the Large Lipid Transfer Protein (LLTP) family. It is best known as a yolk protein that provides nutrients to the eggs during oogenesis. However, studies have shown that Vg is also associated with functions related to antibacterial, antiviral, and oxidative stress protection. In honeybee workers, Vg plays a role in regulating lifespan and social behaviors. However, the molecular basis of these phenotypes are not yet understood. Recent structural work combining AlphaFold modelling with cryo-EM revealed the position of the von Willebrand factor domain (vWF) in honeybee Vg. The vWF domain interacts with multiple proteins and has ligand-binding capacity and interactions with immune systems. To fully characterize and understand the role of the vWF domain, a well-functioning bacterial expression system yielding folded vWF must be developed.

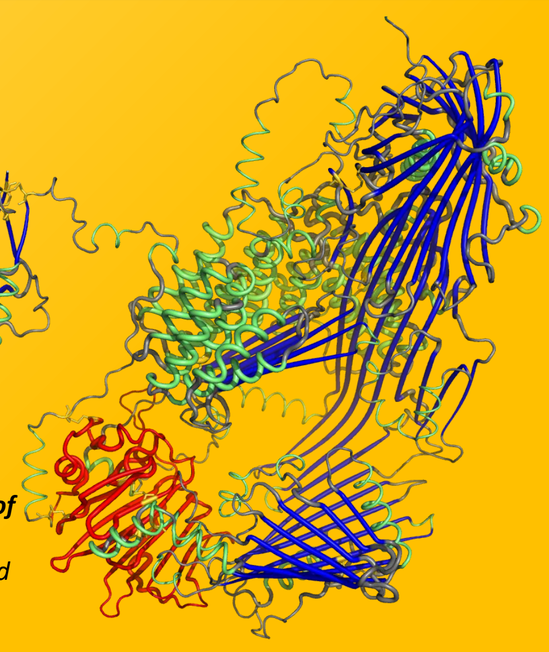
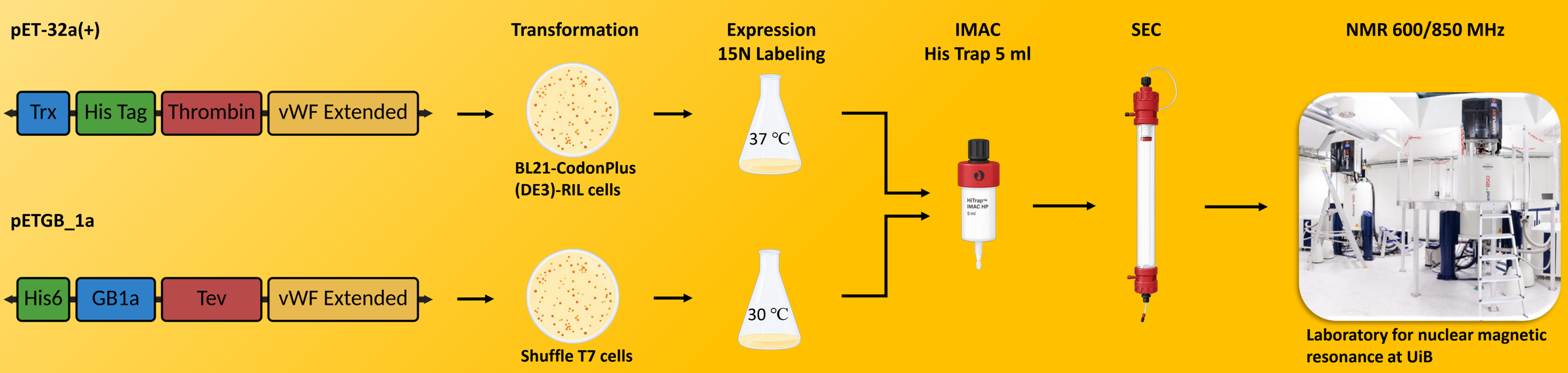


Figure 1: Predicted structure of honeybee vitellogenin. vWF domain shown in red. *Adapted from Leipart et al. (2022) with PyMol.*

Method



Results

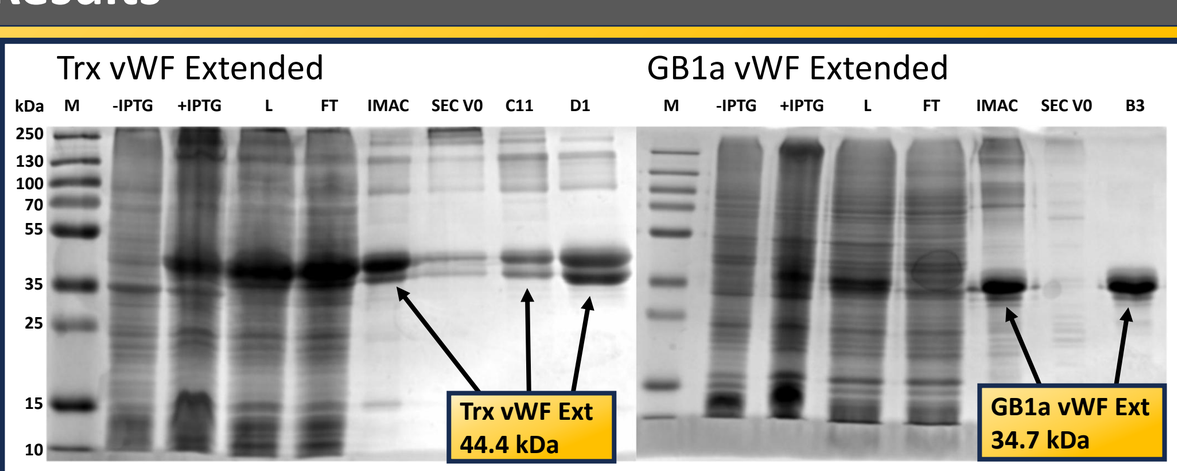


Figure 2: Expression and Purification of vWF Extended proteins analyzed by SDS-PAGE*. Both constructs were expressed after IPTG induction, and the expressed proteins were recovered with IMAC followed by SEC purification. The final fraction (B3) from GB1a construct have higher purity than fraction (D1) from Trx construct. M = molecular weight marker; −IPTG/+IPTG = before/after induction; L = lysate; FT = IMAC flow-through; IMAC = HisTrap elution; SEC V0 = SEC void volume; C11, D1 and B3 = SEC elution fractions.*

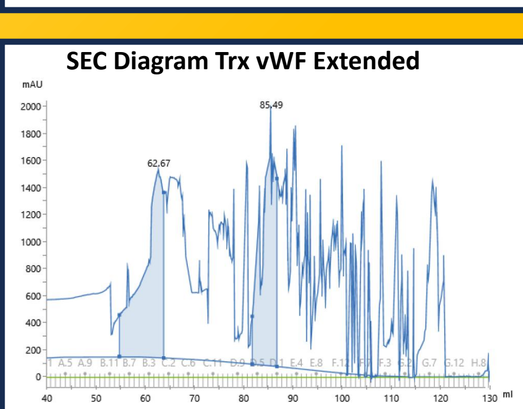


Figure 3A: Size Exclusion chromatography diagram of Trx vWF Ext protein. *Trx vWF Ext monomer eluted around 62 ml which was expected according to elution profile. However, the signal is weak and broad all the way to 100+ ml. That profile could indicate a protein with poor long-term stability and multiple misfolded states.*

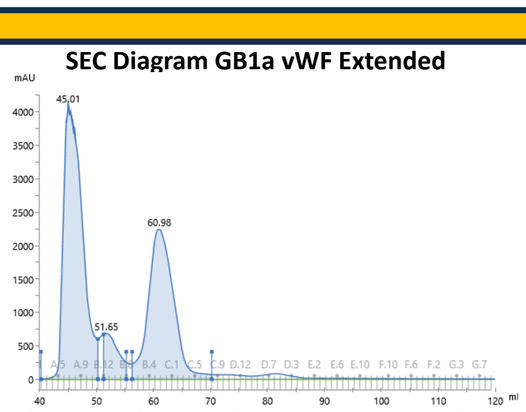


Figure 3B: Size Exclusion chromatography diagram of GB1a vWF Ext protein. *GB1a vWF Ext eluted at 61 ml which is before the expected 68 ml. The SEC profile is clean with distinct peaks with strong signals. The peak at 61 ml could mean a protein with higher hydrodynamic radius, but SEC cannot conclude which state the protein was in.*

Table 1: Total yield of Trx/GB1a vWF Ext protein after purification and NMR preparation. *The GB1a construct and Shuffle T7 cells improved the yield of protein by 12.7-fold in molar concentration. 520 µM of GB1a vWF Ext is within range of what an NMR analysis needs.*

Construct	Final concentration	Final molar concentration	Fold increase
Trx vWF Extended	1.84 mg/mL	41 µM	1.0×
GB1a vWF Extended	18.11 mg/mL	520 µM	12.7×

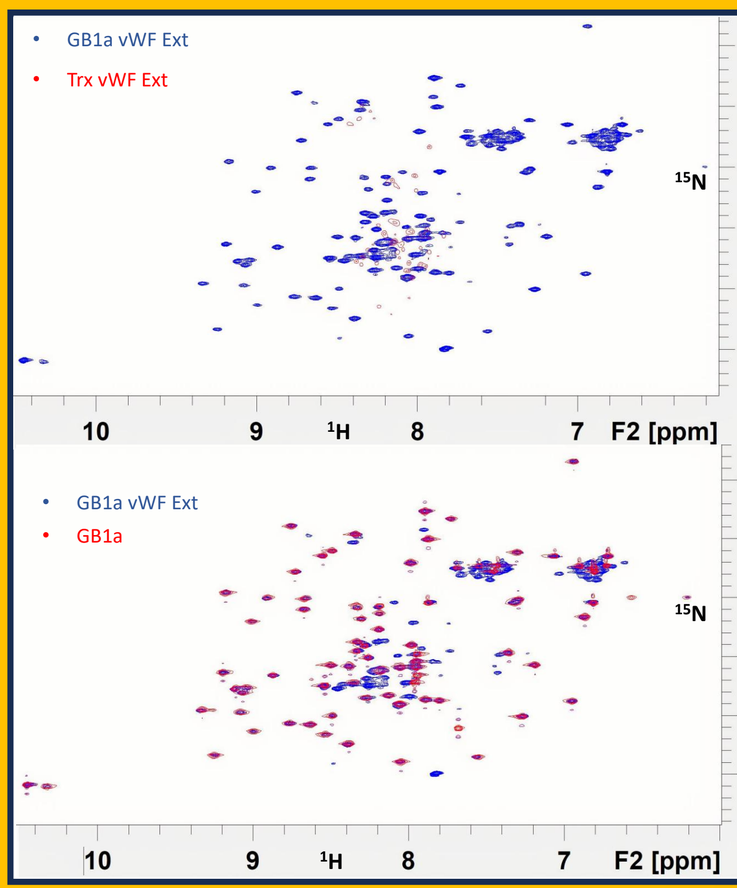


Figure 4A: $^{1}H^{15}N$ HSQC at 600 MHz of GB1a vWF Ext in blue overlayed TROSY 850 MHz of Trx vWF Ext in red. *GB1a vWF Ext in blue shows strong 2D spectrum compared to Trx vWF Ext in red. This was expected as 41 µM concentration of Trx vWF Ext was too low. The GB1a vWF Ext has good dispersal of signals, except two clusters which represents side-chains. However, a protein at 35 kDa should have far more signals than observed here.*

Figure 4B: $^{1}H^{15}N$ HSQC at 600 MHz of GB1 vWF Ext in blue overlayed HSQCETF3GPSI 600 MHz of GB1a in red. *This 2D spectrums shows high degree of overlaps between GB1a and GB1a vWF Ext signal. That suggest that most well defined signals originates from the GB1a region. The GB1a region appears well folded and stable while vWF Ext region appears to have some partial misfold or disordered regions because of the two clusters observed and the weaker signaling.*

Conclusion

- Shuffle T7 cells and pETGB_1a plasmid improved the total yield by ~12.7-fold for vWF Extended compared with BL21-CodonPlus (DE3)-RIL cells and pET-32a(+) plasmid. Shuffle T7 cells supports disulfide bond formation in the cytoplasm which is suitable for vWF Extended.
- The NMR spectra of Trx vWF Ext shows that low sample concentration strongly affects the signals received in the NMR 2D spectra. The purification and NMR of Trx vWF was only done once here which means there are not enough data to conclude if this construct could work or not.
- GB1 vWF Ext produced NMR 2D spectra with good dispersal of signals, but the overlap of signals with GB1a alone suggest that further optimization of the GB1a vWF Ext construct must be done to fully characterize the vWF domain.
- One interesting approach that could be tested is deuterization with D2O of the expression medium which could improve signal strength.

References

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