

Case Study – GFPuv



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PROTEIN SOLUTIONS

Introduction

The green fluorescent protein (GFP) is found in jellyfish and is involved in bioluminescence. It absorbs UV light and glows green which has made it extremely useful in scientific research. The molecule is composed of 238 AA and weighs 26KDa with a pI of 6.5. The structure contains 2 non-bonded cys and the protein is tubular with 11 beta sheets enclosing an irregular alpha-helix which provides scaffold for a central chromophore.

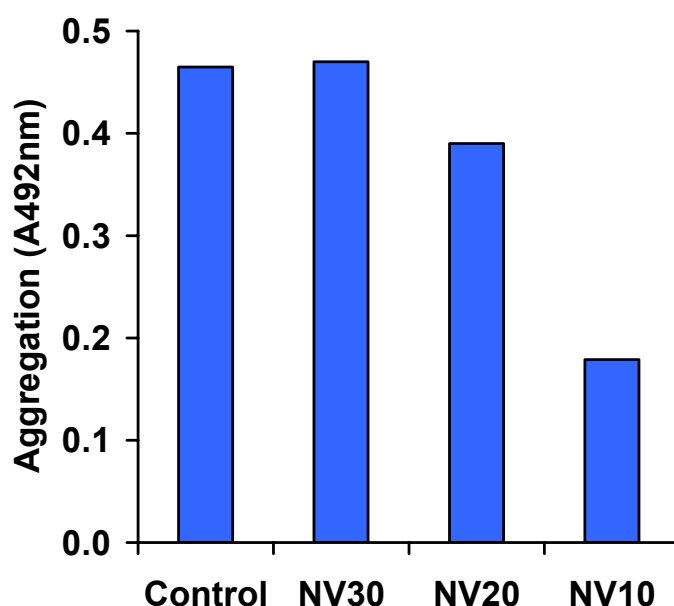
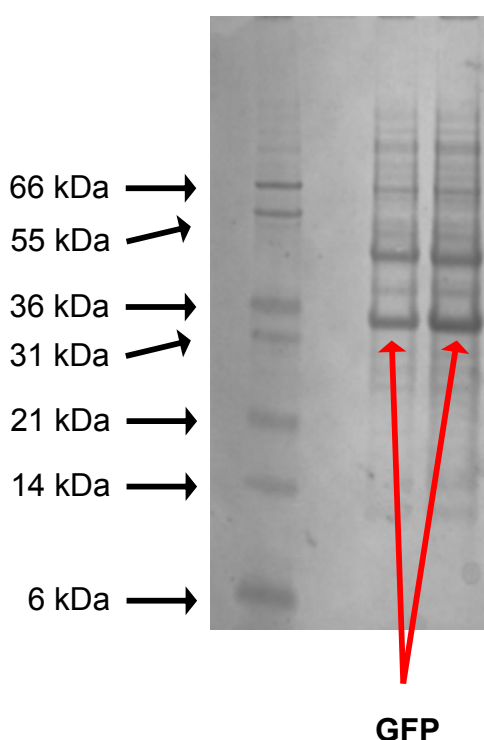
Summary

Expedeon's protective agents, NV10, NV20 and NV30 all significantly improved refolding of GFPuv. NV10 was most effective, suppressing aggregation by over 60%. Refold yields increased significantly using each protectant, NV10 increasing the yield by >500% with a corresponding 5-fold improvement in activity during refolding from a crude inclusion body preparation.

Expedeon has developed a novel, proprietary technology that is radically improving the processing of proteins. Our NVoy Polymer technology provides a simple method that eliminates the need to screen multiple conditions whilst reducing yield losses due to aggregation and allowing higher protein concentrations to be analysed. It's generic use allows a Yes/No answer to be reached quickly on refolding success and the technology is scaleable for low cost processing.

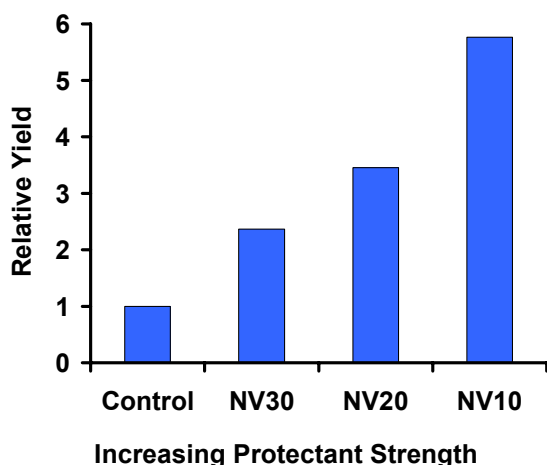
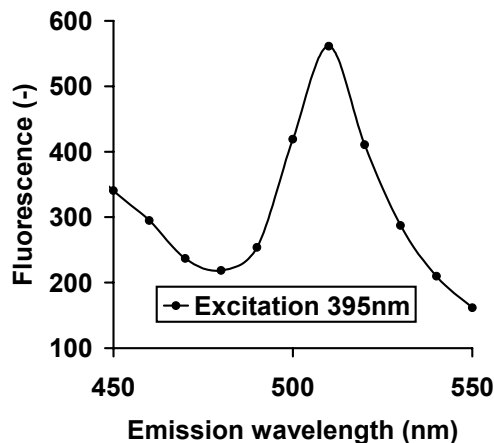
Refold Methodology

Unpurified inclusion bodies were solubilised using 6M GnHCL, 50mM MES, pH 5.5 denaturation buffer. Denatured protein at 4.9mg/ml was then refolded using a 50mM Tris buffer, pH 8.0, and 3 formulations of Expedeon's protective agents, NV10, NV20 and NV30. The three different strength formulations were used to suppress aggregation of protein and replace commonly used additives such as arginine and detergents. Their controlled release allows protein to refold correctly – obvious differences were observed when the formulation was varied with the weakest aggregation suppressor, NV30, having little effect. As the strength of the formulation increased, aggregation was suppressed. The medium strength, NV20, and high strength, NV10, protectants reduced aggregation by 16% and 61% respectively.



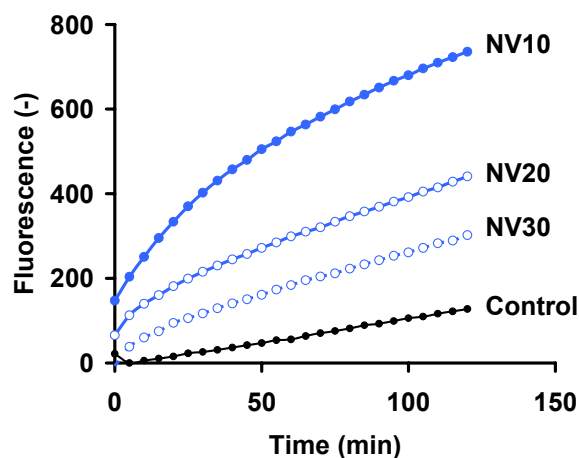
Results

GFP is fluorescent in the native form but loses this ability when denatured. The central chromophore becomes fluorescent relatively late in the folding pathway of GFP. Therefore, fluorescence measurements can be used to determine the kinetics and extent of refolding for this protein. Refolded GFP showed the emission peak on the right when excited with light at 395nm - characteristic of fully folded GFP.



The fluorescence yields were compared to the control sample with no NV protectant to determine relative refolding yields after 120 min. The results show that refolding yield improves as the protectant strength increases with a maximum improvement of 580% in the presence of NV10.

The refolding kinetics indicate that the total fluorescence yield is always higher than the control sample when the NV protectants are added. This is because the aggregation levels are lower in the presence of the protectants. Therefore, a greater pool of monomeric protein undergoing refolding exists at any given time since the irreversible losses due to aggregation are minimised.



Conclusion

Expedeon's Refold Master kit, incorporating NVoy Polymer technology, greatly reduces aggregation losses and allows refolding from a crude inclusion body extract. In addition, the reagents are compatible with most analysis techniques and downstream processing methods. The ability to suppress aggregation but allow successful refolding enables experiments to be performed at high protein concentration and with more aggregation prone variants than GFPuv such as wild-type GFP.

References

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