Reverse engineer blue fluorescent peptide by site-directed mutagenesis of iGFP

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INTRODUCTION

Green fluorescent protein (GFP) is a commonly used protein reporter with a wide range of applications as a fusion tag for proteins of interest.[1] However, its relatively large size (26.9 kDa) can prevent a protein of interest tagged with GFP from passing across cell membranes or interfere with the function of the protein of interest, particularly if it is similar in size to GFP.[1] A small (~10.5 kDa) green fluorescent peptide (iGFP) was developed by Dr. Jonathan Zhang from the chromophore sequence of full-length GFP. Using the GFP derivative BFP as a model, we are developing a small blue fluorescent peptide (iBFP) by site-directed mutagenesis of iGFP at a site that was rationally chosen. This peptide, produced in *E. coli*, would share iGFP's diminished effects on the protein of interest due to size while maintaining its fluorescent properties. Therefore, its potential applications are similar to those of full-length GFP and iGFP as a genetically encoded molecular probe with unique fluorescence.[1]

DESIGN

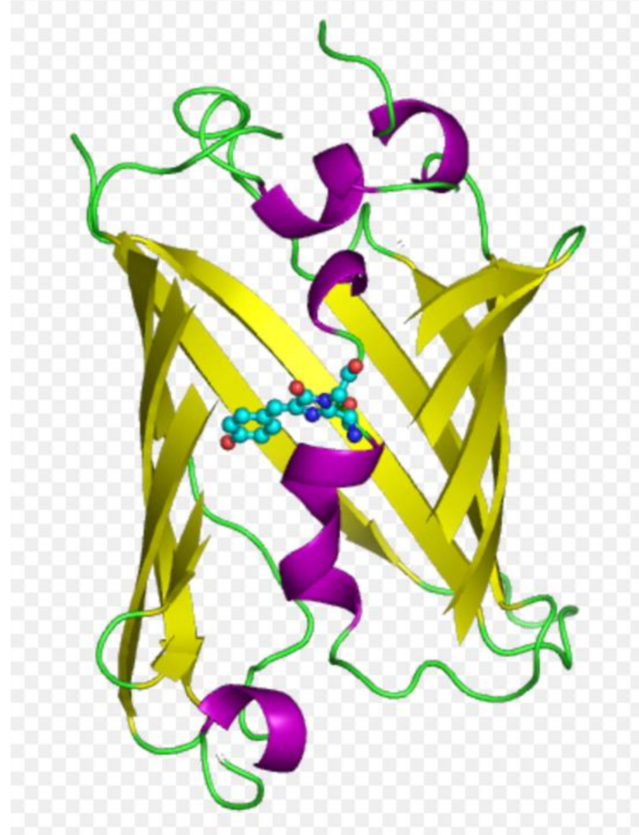

Figure 1. Structure of Green Fluorescent Protein (GFP).[4] Connected central spheres represent internal active site chromophore

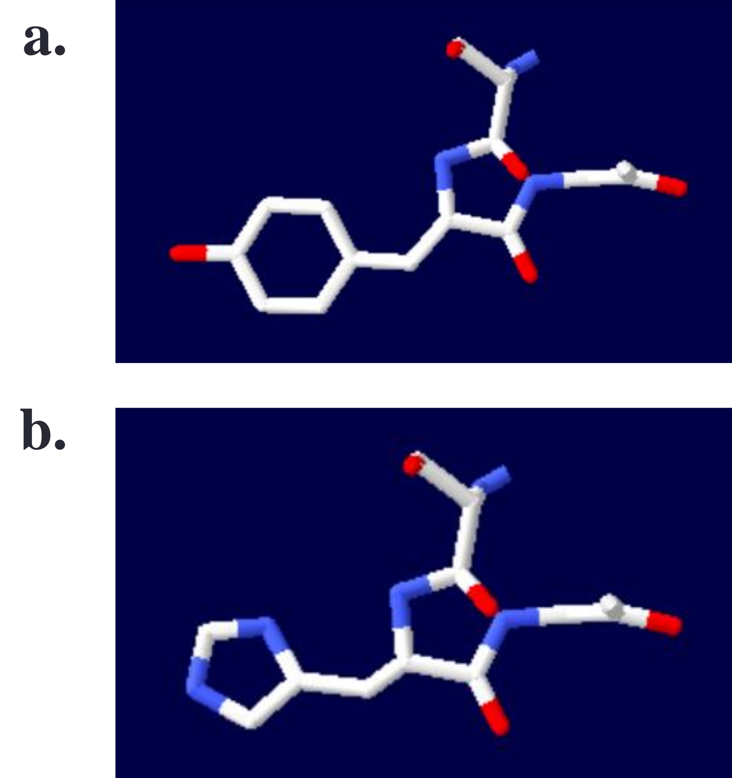

Figure 2. Key chromophore amino acid residues for GFP/iGFP (a) and BFP/iBFP (b).

- GFP and iGFP chromophores are 5-membered imidazolidinone rings formed by amino acid residues Ser65-Tyr66-Gly67 within protein's cylindrical structure[2] (Figure 1)
- Single point Y66H mutation of GFP has been shown to alter the excitation and emission behavior of this chromophore to create BFP[3] (Figure 2)
- Apply Y88H mutation to iGFP through site-directed mutagenesis to create peptide with similarly-shifted fluorescence to BFP (iBFP)

RESULTS

DNA

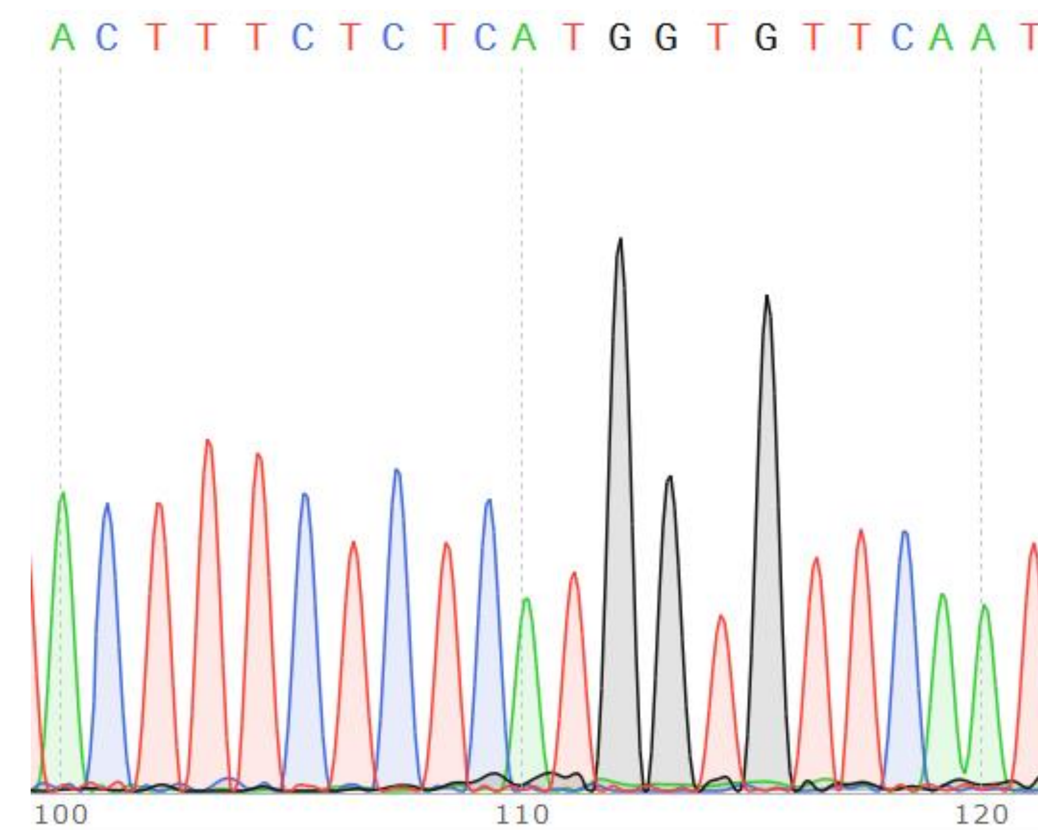

Figure 3. Chromatogram of iBFP DNA sequencing at site of Y66H mutation. Sequencing performed by Sequetech.

Figure 4. Comparison of iGFP sequence to iBFP sequence at site of Y66H mutation. Box surrounds key chromophore amino acid residues for iGFP and iBFP.

PROTEIN

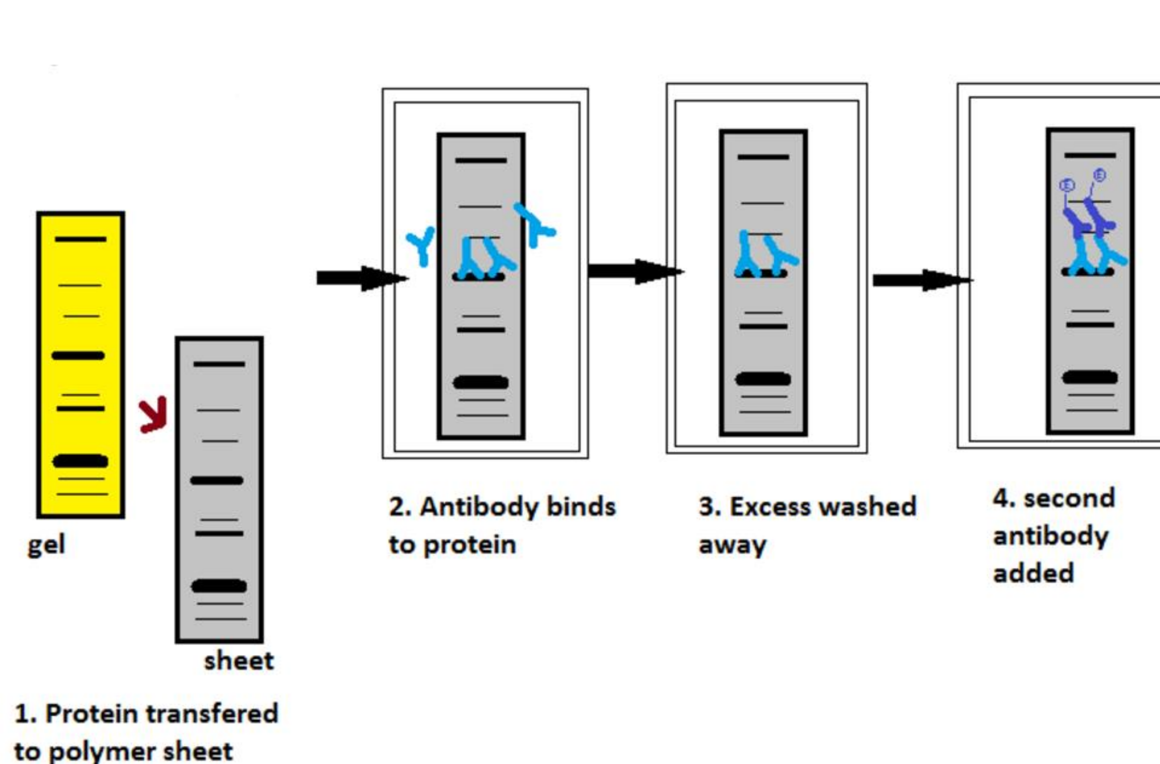

Figure 5. General Western blot protocol.[5] Primary antibody (blue) is specific to protein of interest. Second antibody (purple) is specific to primary antibody and contains substrate for subsequent visualization reaction.

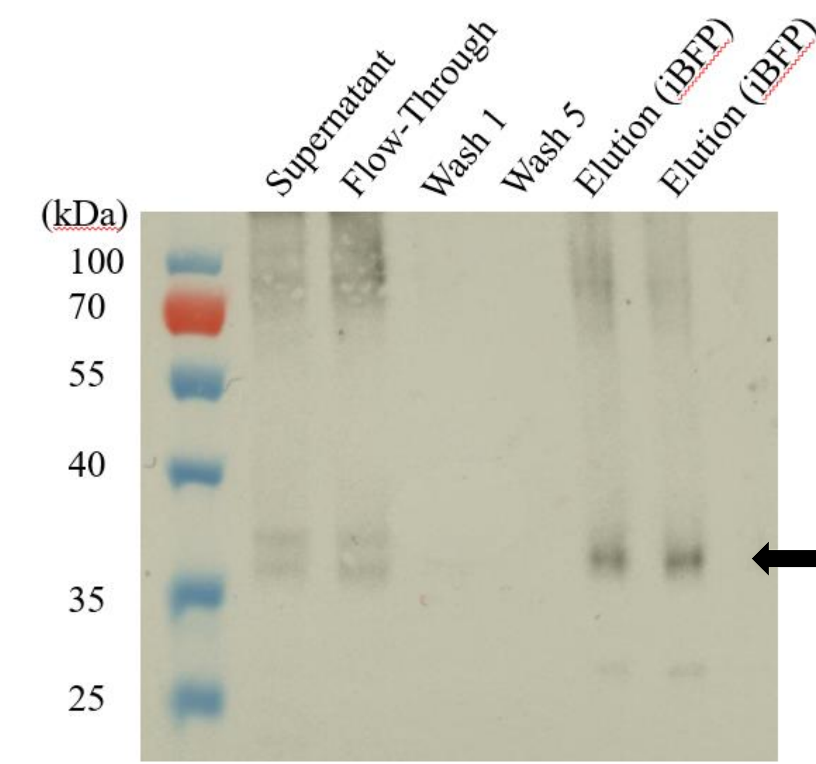

Figure 6. Western blot for iBFP purification. Each well in initial gel contained one sample from a step (as labeled) in purification of iBFP. Arrow indicates iBFP (predicted size 37-39 kDa.)

CONCLUSIONS

- DNA sequencing results suggest successful Y66H single point mutation of iGFP with no additional mutations (Figures 3, 4)
- Western blot results show protein band at predicted size of iBFP, suggesting successful expression and purification of peptide from *E. coli* (Figure 6)

Future Work:

- Perform assay to characterize fluorescent properties of iBFP
- Transfect plasmid into Hela cells to analyze properties in mammalian cell culture
- Apply methodology of reverse engineering to therapeutic proteins

REFERENCES

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ACKNOWLEDGEMENTS

We would like to thank the 2016 Kuehler Undergraduate Research Program for their support of this project.