Biophysical Analysis of Small Protein Ligands

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Abstract

The Hackel Lab utilizes protein engineering to design novel protein scaffolds as effective affinity reagents. There are many applications of protein engineering in regards to molecular imaging, therapeutics, and biotechnology. Protein scaffolds are designed to maintain structural rigidity and stability while enabling mutation in one or more regions to provide novel or improved function. My objective is to characterize the biophysical properties of native truncated Gp2 scaffold protein ($tGp2_{WT}$), including measurement of the secondary and tertiary structure, thermal and chemical stability, and solubility. The structural and folding attributes of the $tGp2_{WT}$ scaffold can be determined by circular dichroism spectroscopy.

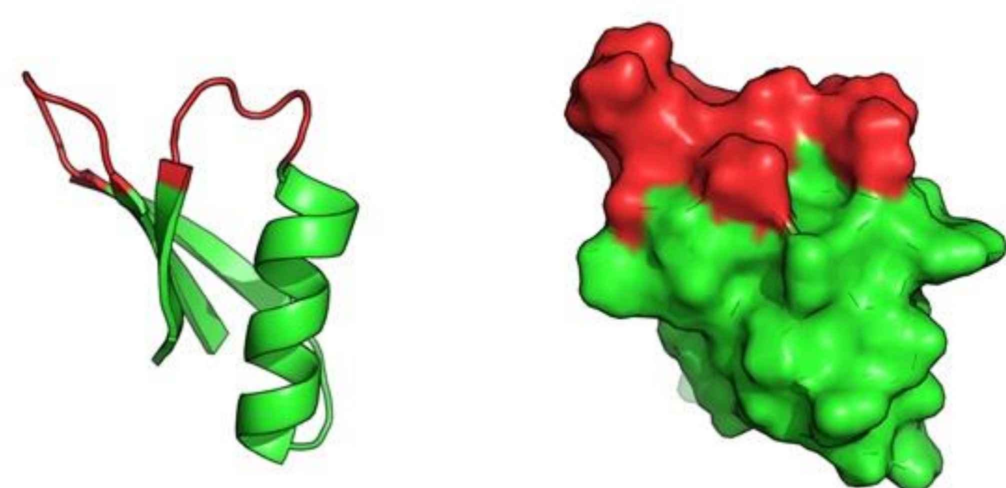

Figure 1: Protein Structure of Parental $tGp2_{WT}$ Scaffold
PDB ID: 2WNM (Image obtained from PyMOL program)
Red indicates the amino acids diversified to engineer novel binding function.

Methodology

$tGp2_{WT}$ protein was produced in JE1 E. coli cells overnight at 37°C and induced for approximately 3.5 hours at 30°C. Similarly, TEV Protease was produced in E. coli cells overnight at 37°C and induced for approximately 3 hours at 30°C.

$tGp2_{WT}$ protein was purified using a polyhistidine-tag (His-tag) and a metal affinity purification column. The protein of interest, fused with a His-tag, binds to the metal resin whereas all other proteins without a His-tag will flow through. The metal resin was eluted with a solution of imidazole, in which the $tGp2_{WT}$ protein will dissociate from the resin. The His-tag was removed using the Tobacco Etch Virus (TEV) Protease which cuts at a specific site of amino acids (TEV Site). After cleavage at the TEV Site, the tagless $tGp2_{WT}$ protein was run through the purification column again. In this second purification run, the ttGp2_WT protein flowed through the column and the TEV Protease, and the remaining His-tag components bind to the column. Further purification of the tagless $tGp2_{WT}$ was done via High-Performance Liquid Chromatography (HPLC). Mass spectrometry (MS) was used to analyze and identify the protein.

Circular dichroism (CD) spectroscopy is a laboratory technique that can determine the various secondary structures of proteins. The α-helix, β-sheet, and loop regions exhibit characteristic CD signatures. CD spectroscopy also monitors the denaturation of the protein through loss of secondary structure. Hence, the secondary structure and stability of the tagless $tGp2_{WT}$ can be determined.

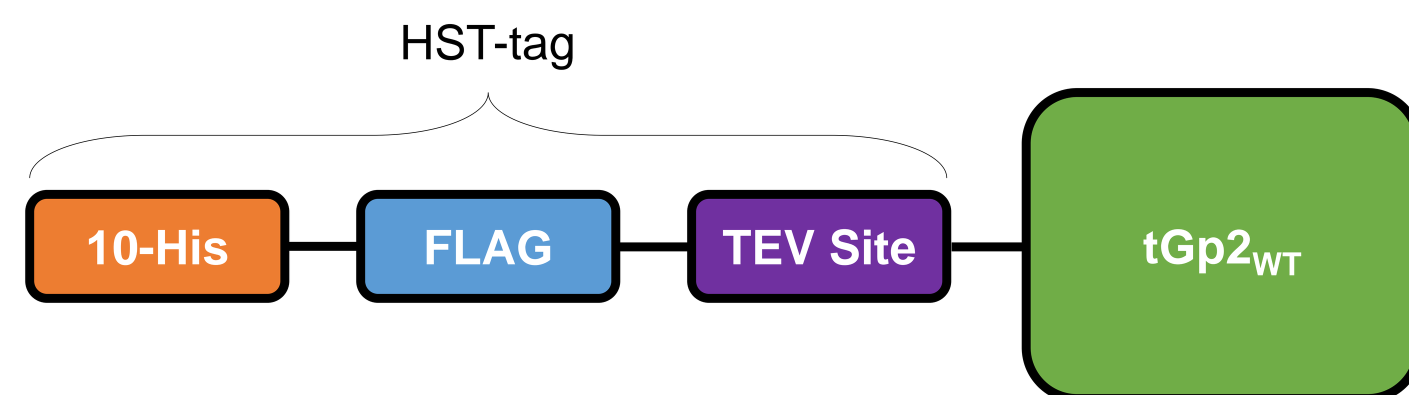

Figure 2: Simple Schematic of the HST_$tGp2_{WT}$ Complex
HST-tag is abbreviated for the (His)_(Solubility)_(TEV Site)-tag and $tGp2_{WT}$ is abbreviated for the truncated, wildtype (or native) Gp2. FLAG-tag is a hydrophilic octapeptide which increases the solubility of the complex. TEV Protease cleaves specifically at the TEV Site, and allows the HST tag to be separated from the $tGp2_{WT}$ scaffold.

Data & Results

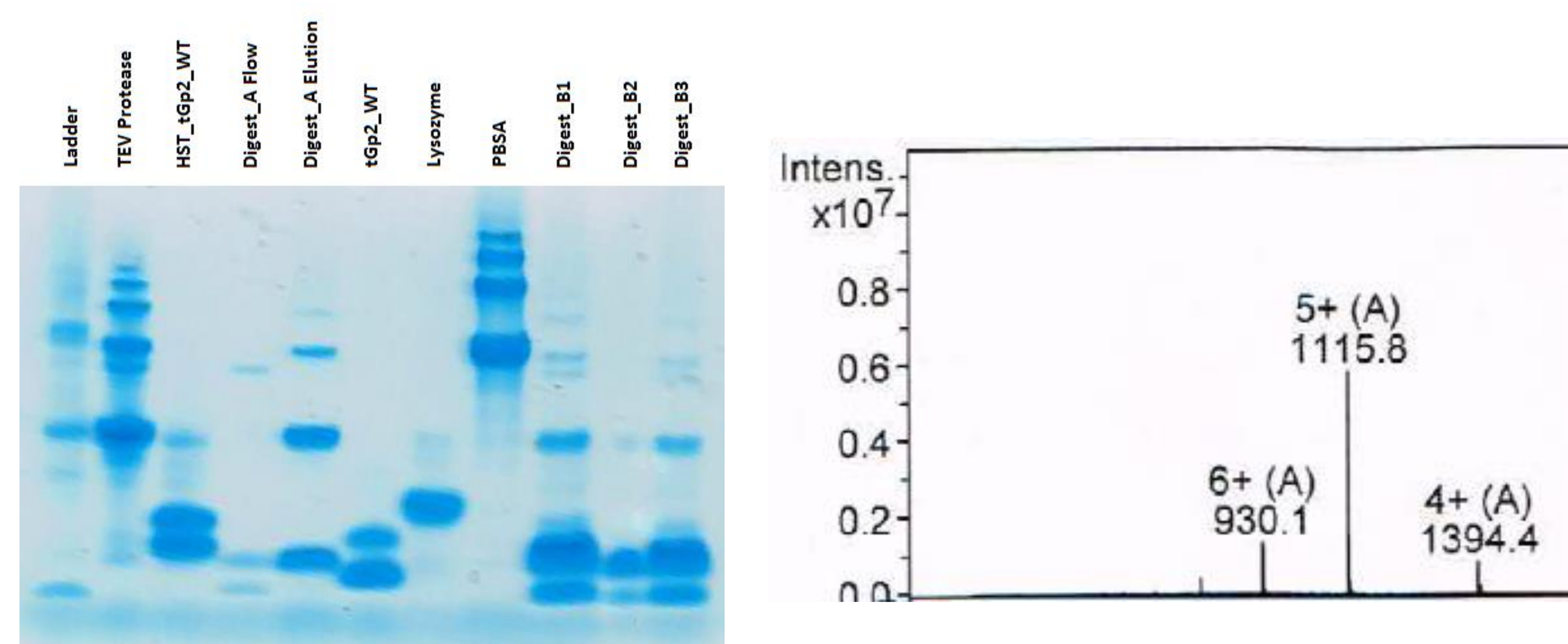

Figure 3: Verification of Tagless Scaffold
Left: SDS-PAGE shows various controls and digestion samples.
Right: Mass Spectrometry of digested protein verifies the correct mass.

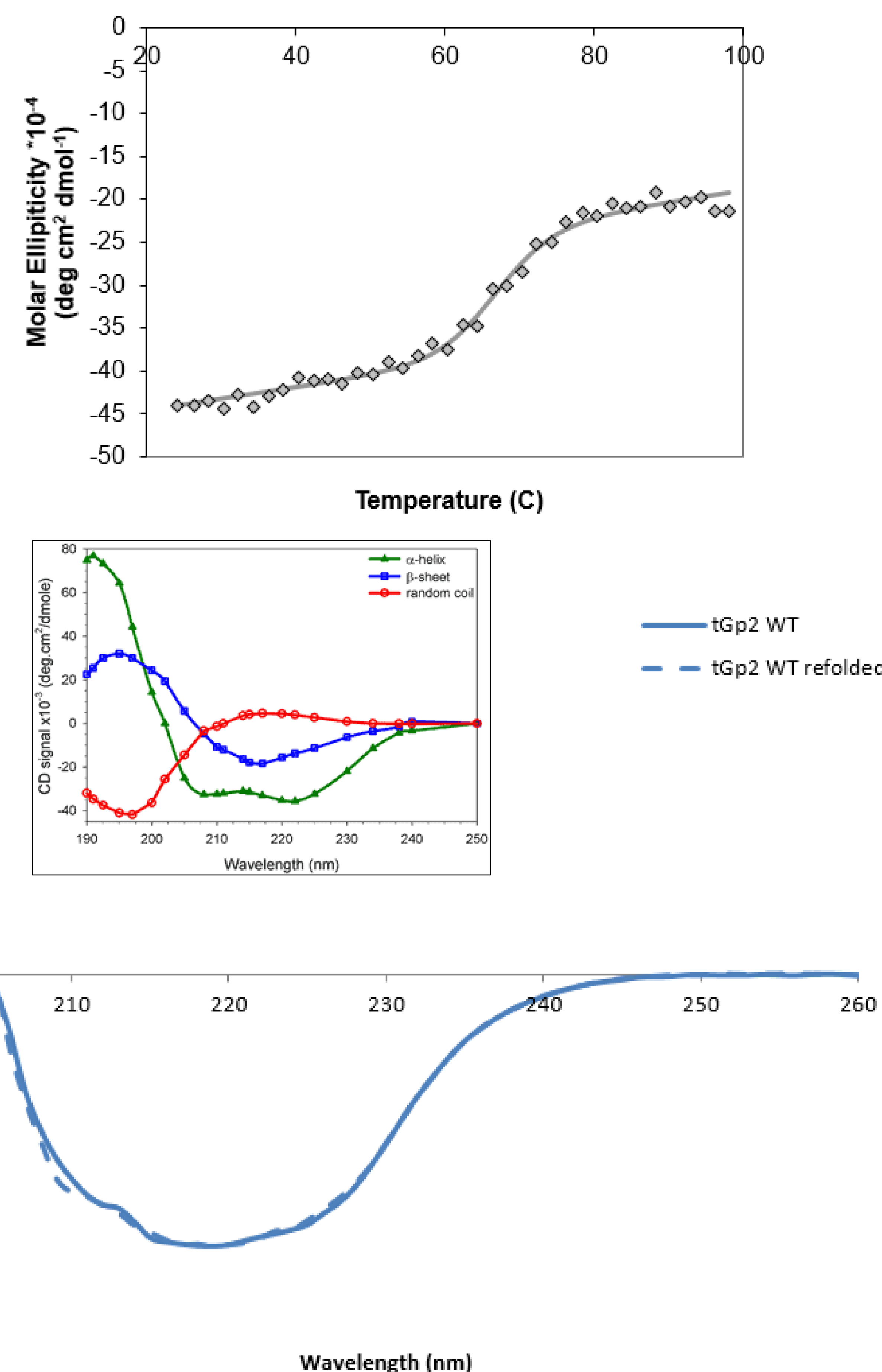

Figure 4: Circular Dichroism Signature of His6_$tGp2_{WT}$
Circular dichroism spectrum used to gauge the approximate make-up of the secondary structure of the folded His6_$tGp2_{WT}$ scaffold and determine the midpoint of thermal denaturation (T_m). Eventually, circular dichroism of the tagless $tGp2_{WT}$ will be compared to the His6_$tGp2_{WT}$ scaffold, to verify whether removal of the tag affects the secondary structure or thermal stability. Inset taken from http://www.fbs.leeds.ac.uk/facilities/cd/images/1.png

Discussion

The HST_ $tGp2_{WT}$ complex is produced, purified, and then cleaved using TEV Protease. This procedure results in tagless $tGp2_{WT}$. An SDS-PAGE was ran using various known controls and digestion samples, and this confirms the tagless $tGp2_{WT}$ protein. However, it is shown that the sample has contaminants! There has been much difficulty in the purification process, and it will be necessary to fully purify through HPLC and other methods.

Mass spectroscopy further verified the digestion product. Tagless $tGp2_{WT}$ was determined at a mass of 5573.9 Da with standard deviation of 0.29. Mass spectroscopy also reveals the contaminant to be a mass of 7797.7 Da with standard deviation of 0.43. Further identification will be done to determine the contaminant.

Circular dichroism verifies that the His6_$tGp2_{WT}$ protein remains stable and follows predicted secondary structure. Purification difficulties are present in the tagless $tGp2_{WT}$ protein. Once fully purified, circular dichroism will verify stability and structure similarly for the tagless $tGp2_{WT}$ protein.

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