Long Assignment 2C: Temperature and Chemical Denaturation

In the third experiment on SNase K63E-E67K, we compare the thermal stability (T_m) through temperature denaturation and the thermodynamic stability (ΔG) through chemical denaturation for mutant and wild-type SNase, assessing the impact of the mutation on the stability of the protein. We also assess/compare the impact of increasing salt concentration on both stability metrics (ΔG and T_m) and the cooperativity of unfolding for chemical denaturation (m-value). Temperature denaturation involves iteratively raising the temperature of the protein solution and measuring the fluorescence of Trp-140 (a residue in SNase that is buried in the folded state and exposed in the unfolded state) at each temperature. The fluorescence values indicate the relative proportions of folded and unfolded SNase. Similarly, the chemical denaturation involves iteratively increasing the concentration of GdnHCl, which unfolds SNase, and measuring the Trp-140 fluorescence at each concentration. We extract T_m from the absorbance by calculating the temperature at which half of the protein is folded, and we extract $\Delta G°_{H2O}$ (the Gibbs free energy of unfolding in standard conditions, with $[\mathrm{GdnHCl}] = 0$) by determining the ratio of concentrations of folded and unfolded states (K_{eq}) at several points along the denaturation, calculating ΔG at these points (using $\Delta G = -RT \ln K_{eq}$), and extrapolating $\Delta G°_{H2O}$ from the well-documented linear relationship between ΔG and [GdnHCl]. Using these metrics, we can quantitatively measure the stability of SNase.

Both denaturation experiments were performed on two SNase solutions: mutant and wild-type, in two experimental conditions: 0.1 M KCl and 1 M KCl. The thermal denaturation curves are presented in Figures 1-2, the chemical denaturation curves in Figures 3-4, the ΔG plots in Figures 5-6, the T_m values in Table 1, and the m-values in Table 2. Overall, the T_m values suggest that the mutation minimally changed the temperature stability of SNase, while the ΔG

values indicate that the mutation decreased its chemical stability. From Table 1, the T_m values of the variant are within 1 °C for both salt conditions, supporting the temperature stability conclusion. Meanwhile, the $\Delta G°_{H2O}$ values (the y-intercepts on the trendlines in Figures 5-6) for the variant were 1.7 kcal/mol higher in 0.1 M KCl and 0.8 kcal/mol higher in 1 M KCl. Since ΔG varies with the log of K_{eq}, these values correspond to the ratio between the concentrations of folded and unfolded protein being over an order of magnitude lower in the mutant at 0.1 M KCl and almost an order of magnitude lower at 1 M KCl. This is a significant difference, indicative of reduced stability of the mutant since a substantially larger, relative amount of the unfolded state is present (on the order of ten times as much as the background, in both salt conditions). The m-values (Table 2) are similar between variant and background at both salt conditions, suggesting similar cooperativity of unfolding. The T_m and ΔG values increased for mutant and background with increasing salt, corresponding to an increase in stability, and interestingly, the stability of the variant increased more than the stability of the background (Table 3).

Initially, it was hypothesized that K63E-E67K would reduce the stability of the SNase protein, since this mutation increases the distance of the 63-67 Coulombic interaction. The data from the chemical denaturation supports this hypothesis, while minimal change in temperature stability was observed. Likely, the weakening of the 63-67 Coulombic interaction does reduce the thermodynamic stability of SNase in the mutant, but the extent of this destabilization cannot be discerned in the lower sensitivity temperature denaturation. Additionally, salt stabilized both the mutant and the background. Two mechanisms support increasing stability with increasing salt. First, salt ions screen unfavorable Coulombic interactions that destabilize the protein. Second, salt ions support the hydrophobic effect because salt ions compete with the protein to interact with the solvent, water, increasing protein folding/aggregation (principle of “salting

out”). Interestingly, the mutant observed higher increases in stability with increasing salt than the background. This may be a result of the increased steric clash present in the 63-67 ion pair in the mutant, which could block salt ions from screening this favorable Coulombic interaction, diminishing the destabilizing effects of salt in the mutant relative to the background.

Figure 1:

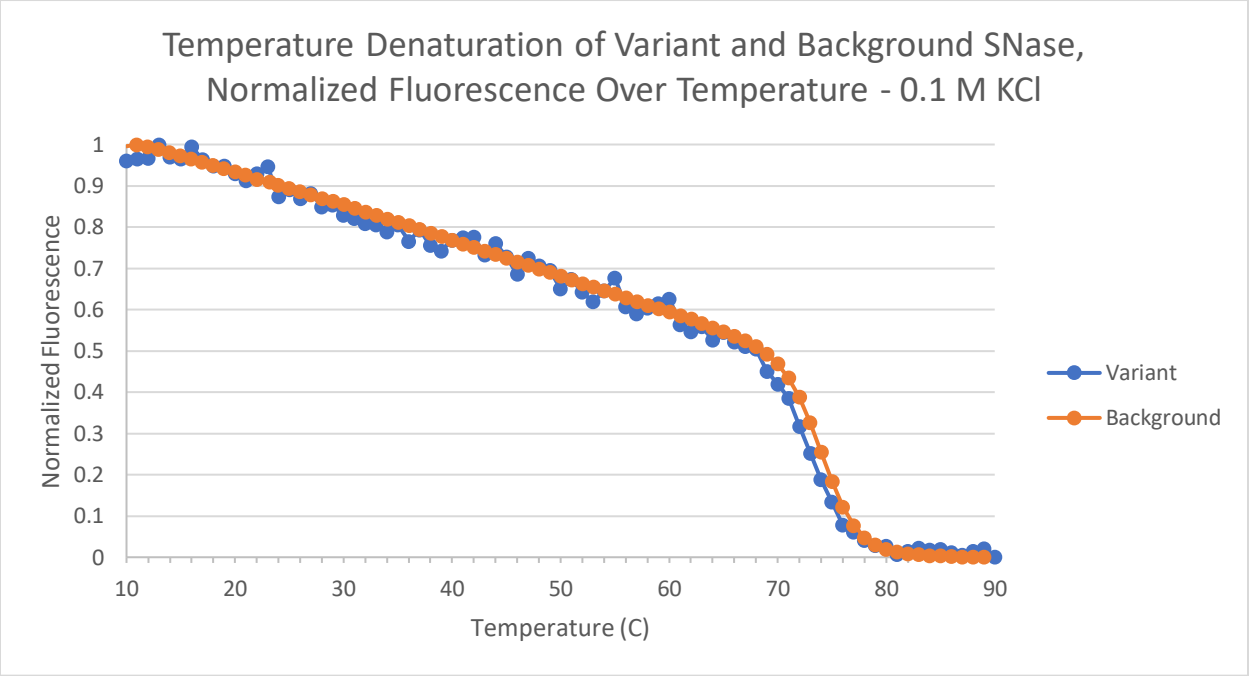

Figure 2:

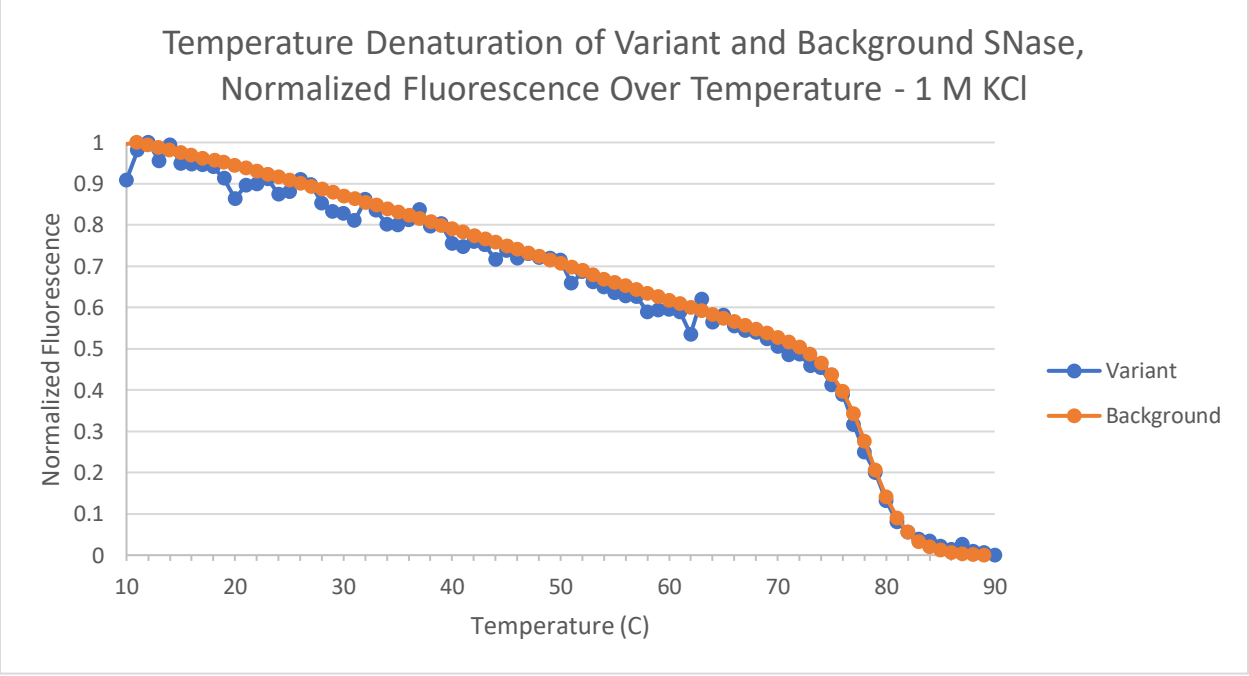

Figure 3:

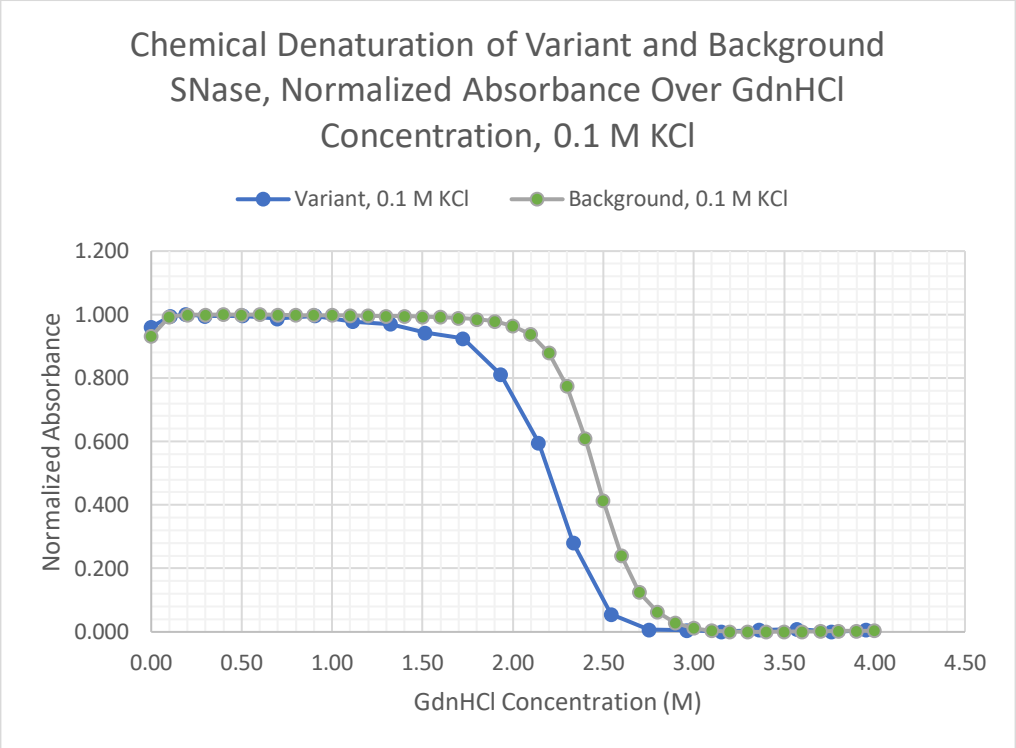

Figure 4:

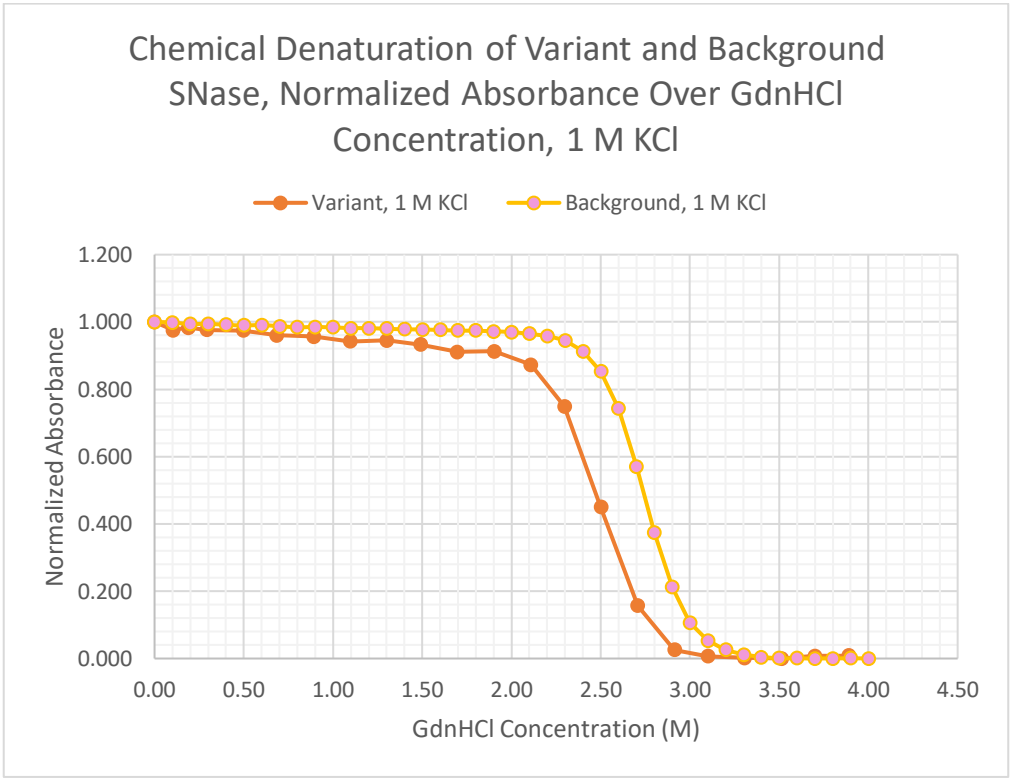

Figure 5:

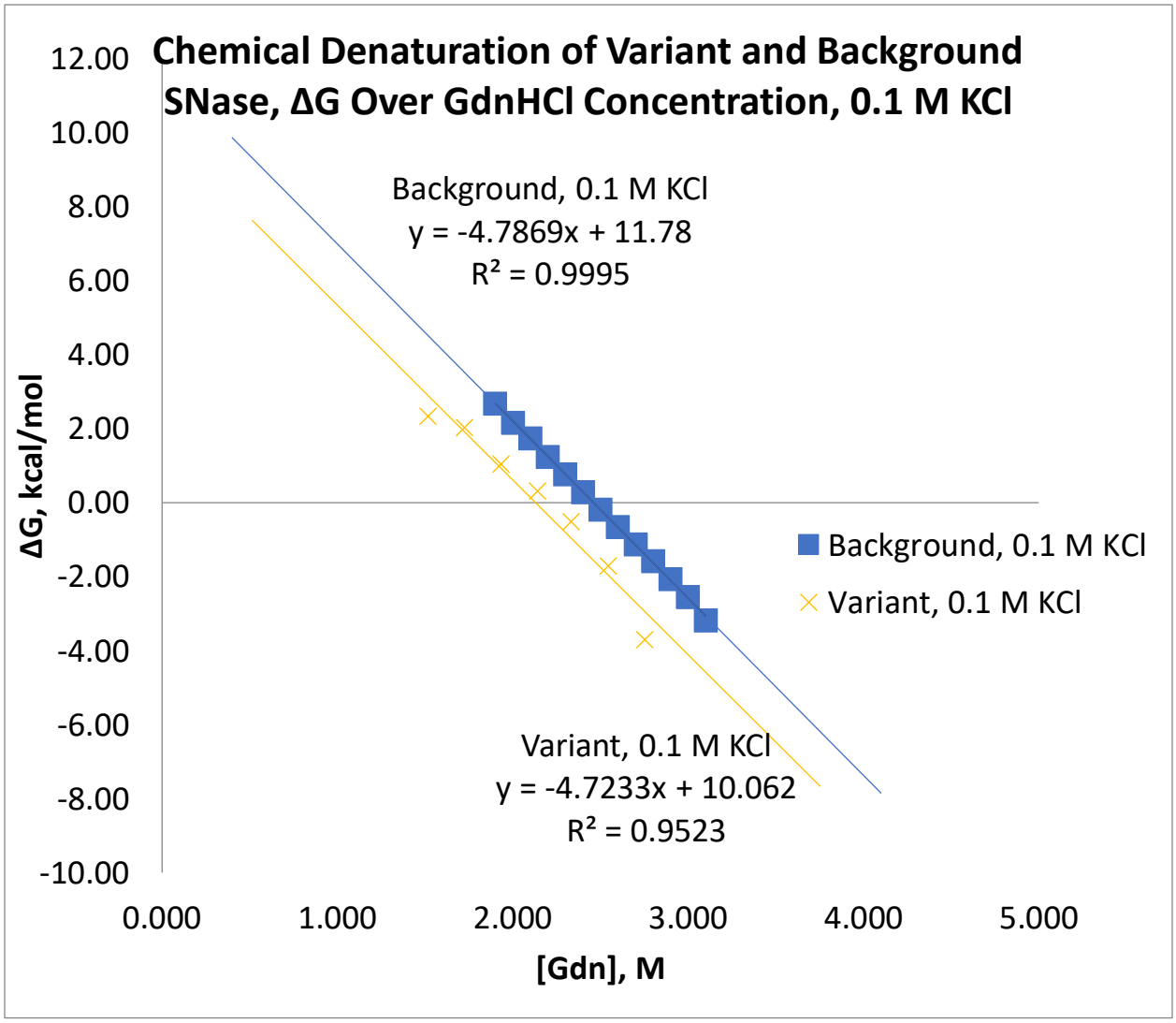
Chemical Denaturation of Variant and Background SNase, ΔG Over GdnHCl Concentration, 0.1 M KCl
Background, 0.1 M KCl
y = -4.7869x + 11.78
R² = 0.9995
Variant, 0.1 M KCl
y = -4.7233x + 10.062
R² = 0.9523
Background, 0.1 M KCl
Variant, 0.1 M KCl
ΔG, kcal/mol
[Gdn], M
12.00
10.00
8.00
6.00
4.00
2.00
0.00
-2.00
-4.00
-6.00
-8.00
-10.00
0.000
1.000
2.000
3.000
4.000
5.000

Figure 6:

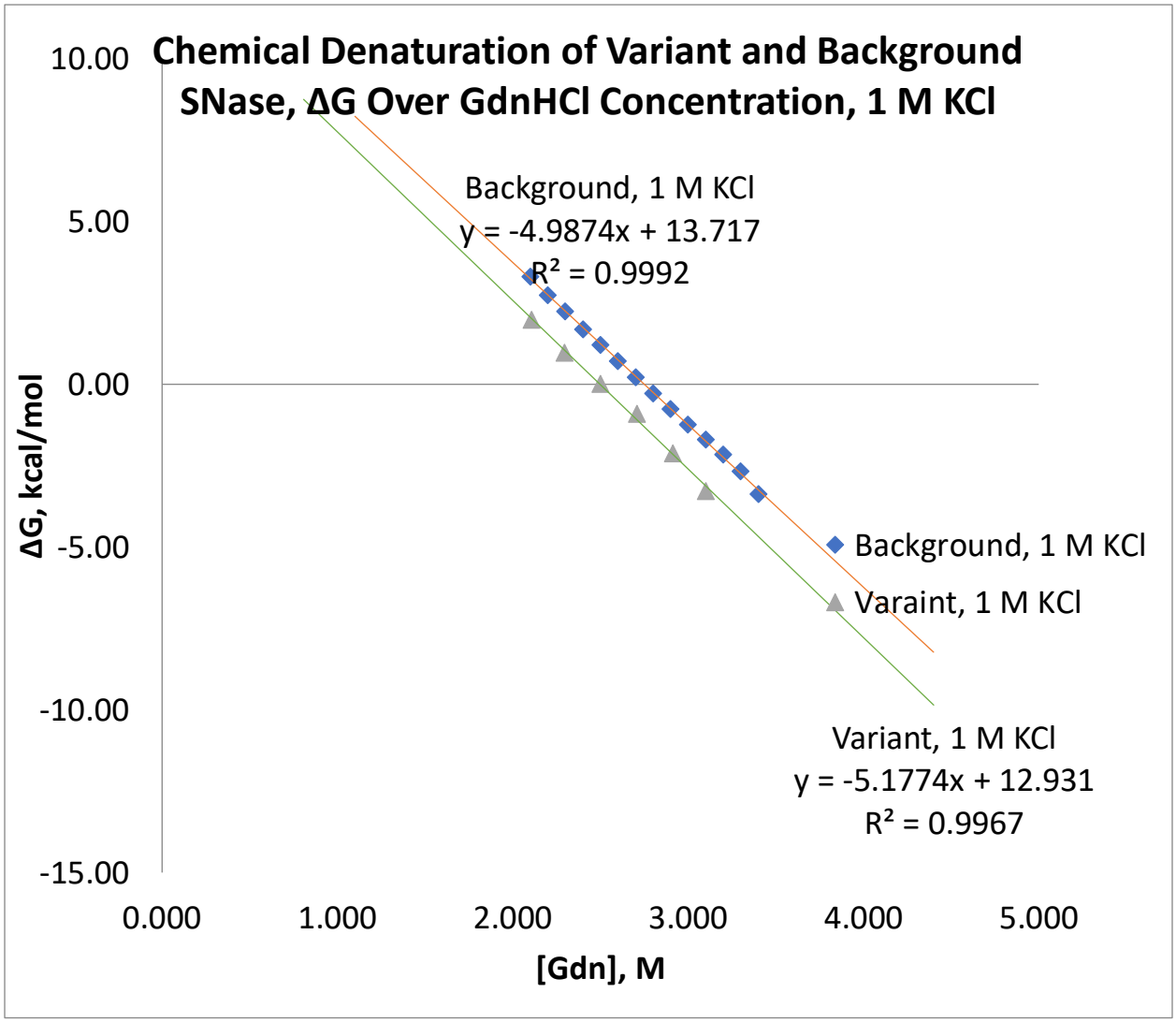

Table 1:

Condition:	Protein:	T_m Value (°C):
0.1 M KCl	K63E-E67K	73.1
0.1 M KCl	Background	74.1
1.0 M KCl	K63E-E67K	78.1
1.0 M KCl	Background	78.4

Table 2:

Condition:	Protein:	m-Value:
0.1 M KCl	K63E-E67K	4.7
0.1 M KCl	Background	4.8

1.0 M KCl	K63E-E67K	5.2
1.0 M KCl	Background	5.0

Table 3:

Condition:	Protein:	ΔT_m Value (°C):	$\Delta\Delta G°_{H2O}$ (kcal/mol)
0.1 M KCl to 1 M KCl	K63E-E67K	5.0	2.0
0.1 M KCl to 1 M KCl	Background	4.3	2.8