Long Assignment 2B: CD Spectroscopy

In the second experiment on SNase K63E-E67K, we compare the circular dichroism (CD) spectra for mutant and wild-type SNase, assessing the impact of the mutation on protein structure. We also qualitatively assess the impact of the mutation on pH and salt stability. CD spectroscopy compares the relative absorbances of left and right circular polarized light of a sample to detect the presence of chiral structures (which absorb polarized light unequally), particularly secondary protein structures. The uneven sum of transmitted light produces an elliptical output polarization, which can be characterized by the degree of ellipticity (mean residue ellipticity, or MRE). By measuring the MRE over a range of wavelengths indicative of secondary structure, we can analyze the structure of SNase by the shape of the spectrum.

CD spectroscopy was performed on two SNase solutions: mutant and wild-type, with two controls: unfolded and denatured (with GdnHCl) SNase, in four experimental conditions: pH 7, 0.1 M KCl; pH 7, 1 M KCl; pH_{mid} ($\approx$ 2.25), 0.1 M KCl; and pH 1, 0.1 M KCl. The unfolded control spectrum is used as a comparison alongside the wild-type to determine how much folded/unfolded character the mutant spectrum possesses to qualitatively infer the structure of SNase at that experimental condition, and the denatured control provides a baseline for complete loss of secondary structure (differing from unfolded, which may still have some residual structure).

The CD spectra are presented in Figures 1-6, and the MRE_{222}/MRE_{208} ratios in Table 1. Overall, the spectra suggest that the mutation minimally impacted the structure of SNase but did increase the pH and salt stability. Examining the pH 7, 0.1 M KCl (standard conditions, Fig. 1) spectra, the shape of the mutant spectrum closely resembles that of background SNase, suggesting similar secondary structure. They both display the characteristic “W” shape indicative

of alpha helix content. However, in the mutant, the minimum at 208 nm relatively lower than the one at 222. From Table 1, the MRE_{222}/MRE_{208} ratio is 11.7% lower for the mutant relative to the background at this condition. This may suggest that there is a slightly higher, relative amount of unfolded mutant at standard conditions compared to background (a reflection of reduced stability). The ratios for the next set of spectra at 1 M KCl (Fig. 2), however, display the opposite. From Table 1, the MRE_{222}/MRE_{208} for the mutant at 1 M KCl (1.234) is closer than the background at 1 M KCl (1.052) to the standard condition background value (1.140). This may suggest that the mutant SNase is slightly more salt stable than the background. For the remaining experimental conditions, the MRE_{222}/MRE_{208} is similar for mutant and wild-type. The most surprising result arose in the pH 2.25 condition (Fig. 3); the shape of the background spectrum has much more unfolded character than the mutant, evidenced by the relatively deep minimum at approximately 206 nm, (halfway between minima of unfolded (204 nm) and folded (208 nm) spectra). In contrast, the mutant retains the characteristic “W” shape of the folded spectrum, suggesting higher pH stability. The final set of spectra at pH 1 (Fig. 4) are both very close in shape to the unfolded, suggesting virtually complete unfolding for mutant and background, as expected in such extreme pH conditions.

Initially, it was hypothesized that K63E-E67K would alter the structure and reduce the stability of the SNase protein, since this mutation increases the distance of the 63-67 Coulombic interaction. The data from this second experiment, however, suggests very minimal change in overall structure and mostly supports the opposite of the hypothesis regarding stability (slightly increased pH and salt stability), though the standard conditions do support the hypothesis (decreased mutant stability). Likely, the Coulombic interaction is not weakened enough to appreciably affect the structure. Furthermore, the mutant has increased steric clash, which may

block the motion of the salt species, thus reducing their screening effect and increasing salt stability. Steric hindrance may also explain the pH stability, though it is unlikely because H+ is a much smaller species. The pH stability result also contradicts the results of the acid unfolding experiment in Lab 10, which suggested decreased pH stability in the mutant. More investigation may be required to draw complete conclusions.

Figure 1:

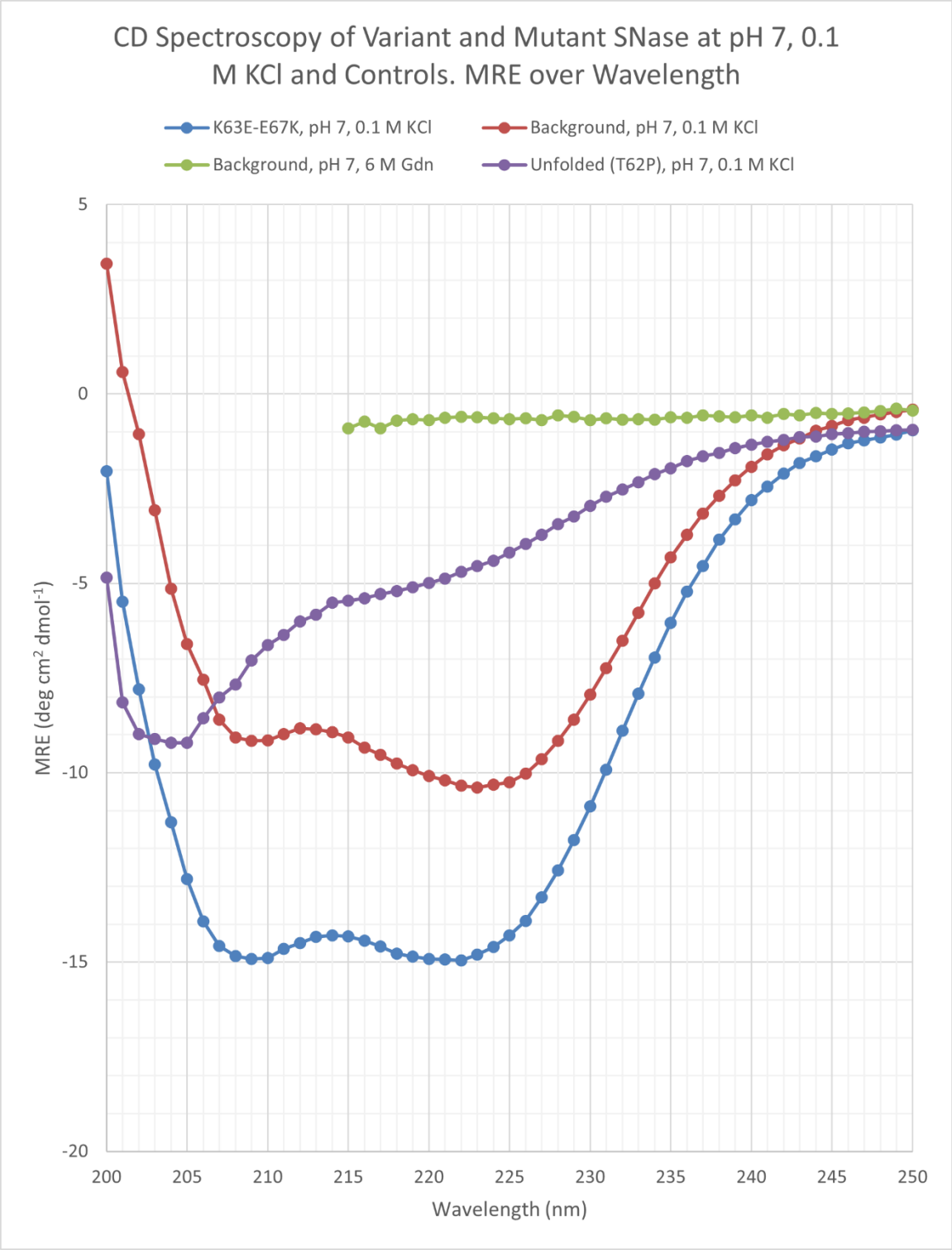
CD Spectroscopy of Variant and Mutant SNase at pH 7, 0.1 M KCl and Controls. MRE over Wavelength
K63E-E67K, pH 7, 0.1 M KCl
Background, pH 7, 0.1 M KCl
Background, pH 7, 6 M Gdn
Unfolded (T62P), pH 7, 0.1 M KCl
MRE (deg cm2 dmol-1)
5
0
-5
-10
-15
-20
200
205
210
215
220
225
230
235
240
245
250
Wavelength (nm)

Figure 2:

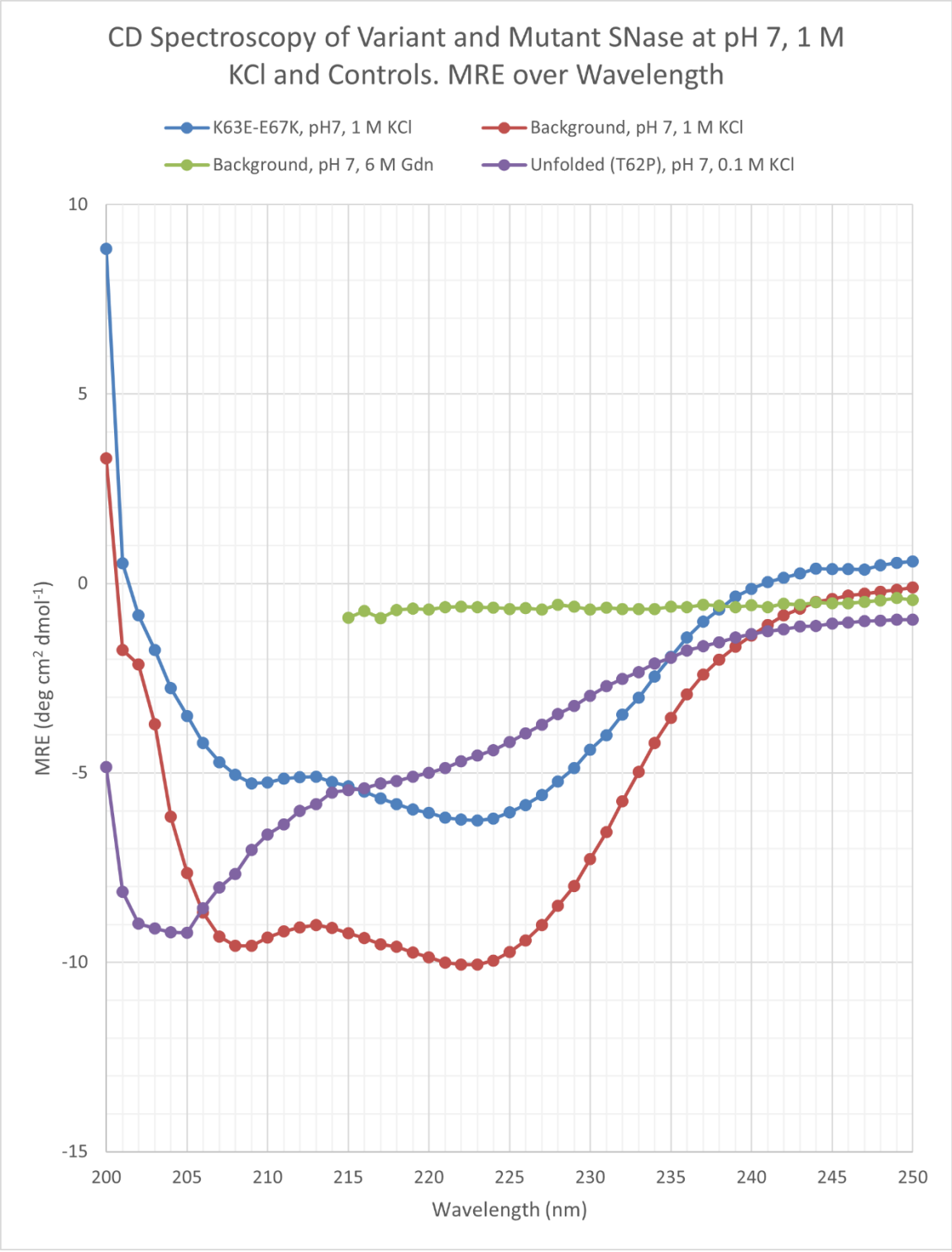
CD Spectroscopy of Variant and Mutant SNase at pH 7, 1 M KCl and Controls. MRE over Wavelength
K63E-E67K, pH7, 1 M KCl
Background, pH 7, 1 M KCl
Background, pH 7, 6 M Gdn
Unfolded (T62P), pH 7, 0.1 M KCl
MRE (deg cm2 dmol-1)
10
5
0
-5
-10
-15
200
205
210
215
220
225
230
235
240
245
250
Wavelength (nm)

Figure 3:

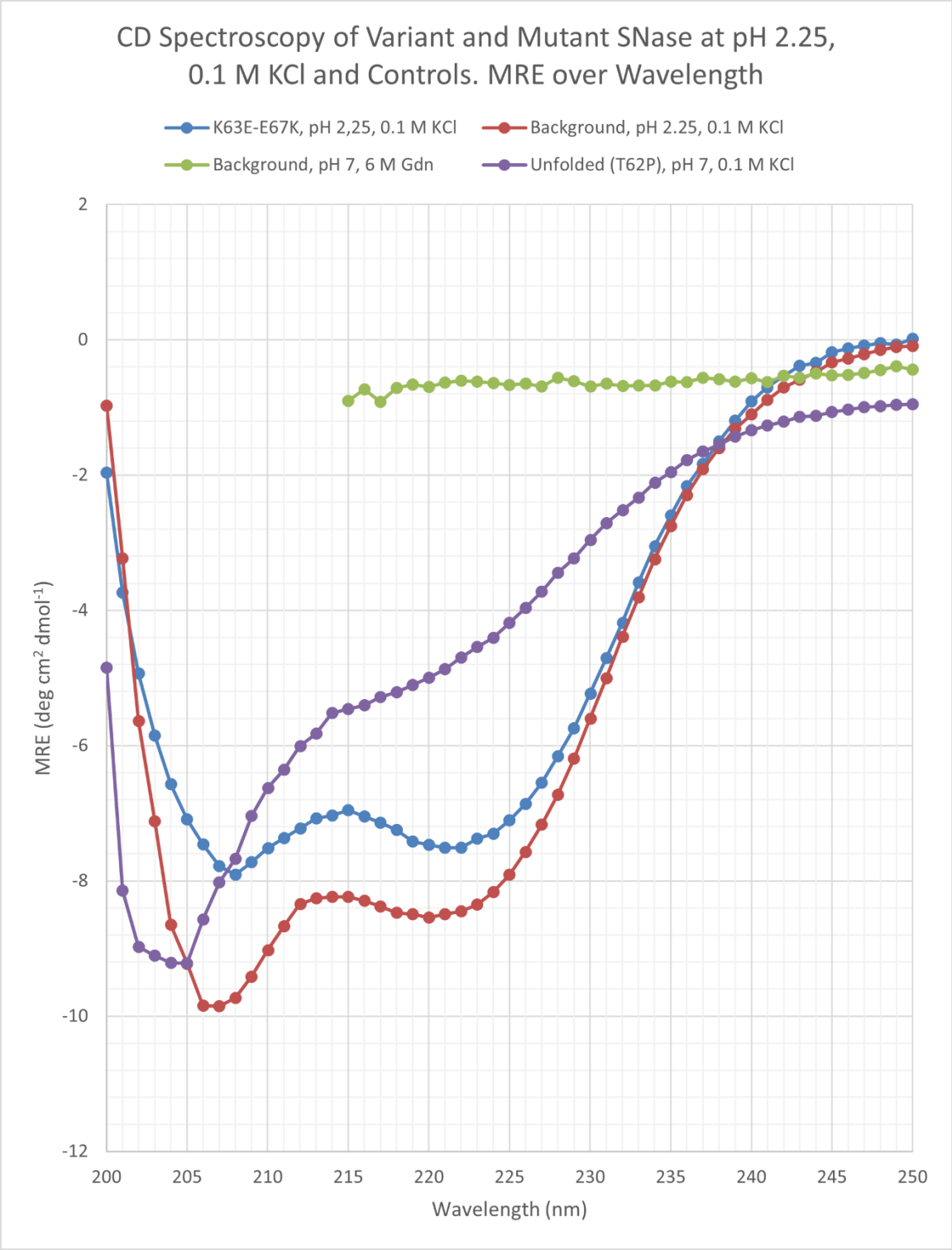
CD Spectroscopy of Variant and Mutant SNase at pH 2.25, 0.1 M KCl and Controls. MRE over Wavelength
K63E-E67K, pH 2,25, 0.1 M KCl
Background, pH 2.25, 0.1 M KCl
Background, pH 7, 6 M Gdn
Unfolded (T62P), pH 7, 0.1 M KCl
MRE (deg cm2 dmol-1)
2
0
-2
-4
-6
-8
-10
-12
200
205
210
215
220
225
230
235
240
245
250
Wavelength (nm)

Figure 4:

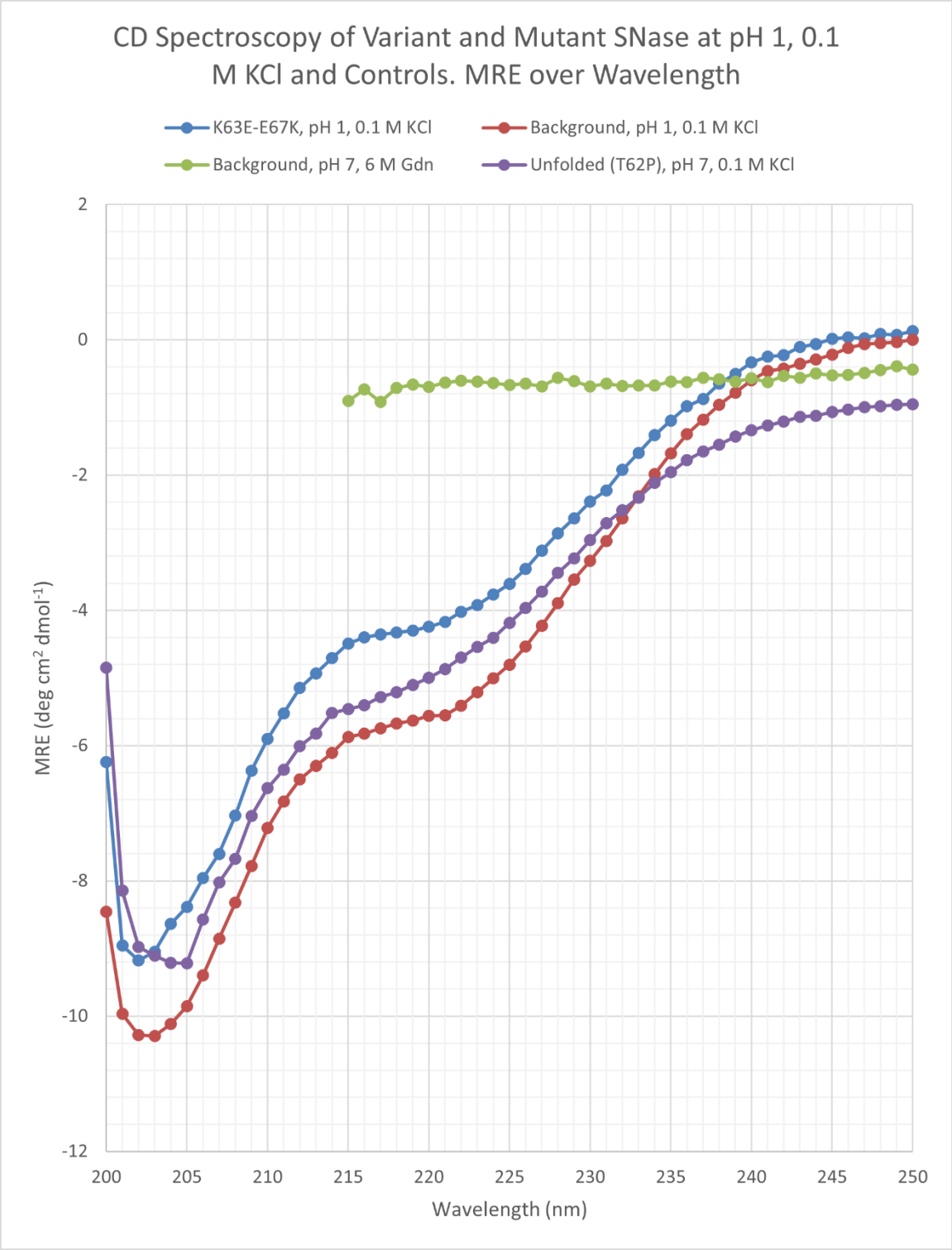
CD Spectroscopy of Variant and Mutant SNase at pH 1, 0.1 M KCl and Controls. MRE over Wavelength
K63E-E67K, pH 1, 0.1 M KCl
Background, pH 1, 0.1 M KCl
Background, pH 7, 6 M Gdn
Unfolded (T62P), pH 7, 0.1 M KCl
MRE (deg cm2 dmol-1)
2
0
-2
-4
-6
-8
-10
-12
200
205
210
215
220
225
230
235
240
245
250
Wavelength (nm)

Figure 5:

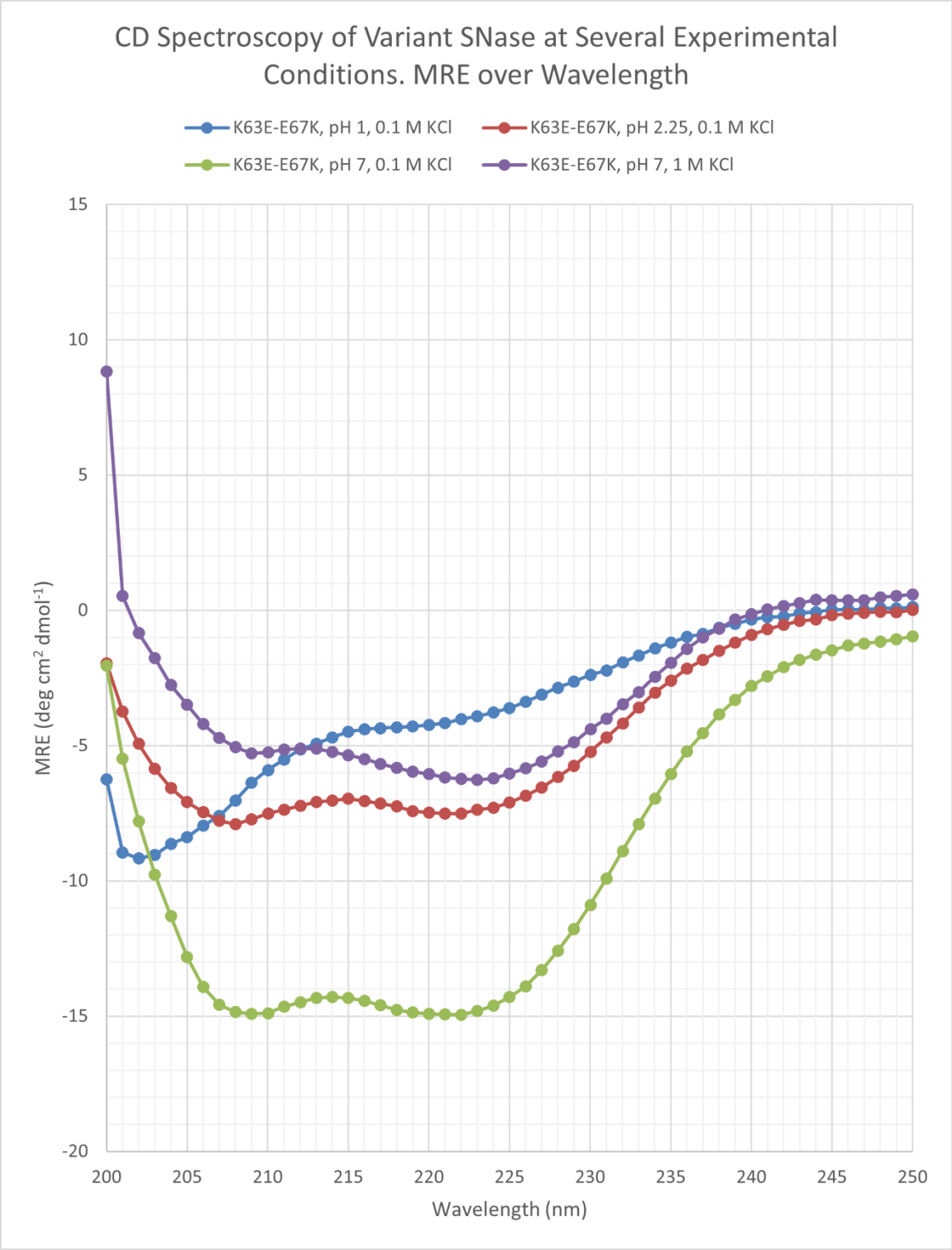
CD Spectroscopy of Variant SNase at Several Experimental Conditions. MRE over Wavelength
K63E-E67K, pH 1, 0.1 M KCl
K63E-E67K, pH 2.25, 0.1 M KCl
K63E-E67K, pH 7, 0.1 M KCl
K63E-E67K, pH 7, 1 M KCl
MRE (deg cm2 dmol-1)
15
10
5
0
-5
-10
-15
-20
200
205
210
215
220
225
230
235
240
245
250
Wavelength (nm)

Figure 6:

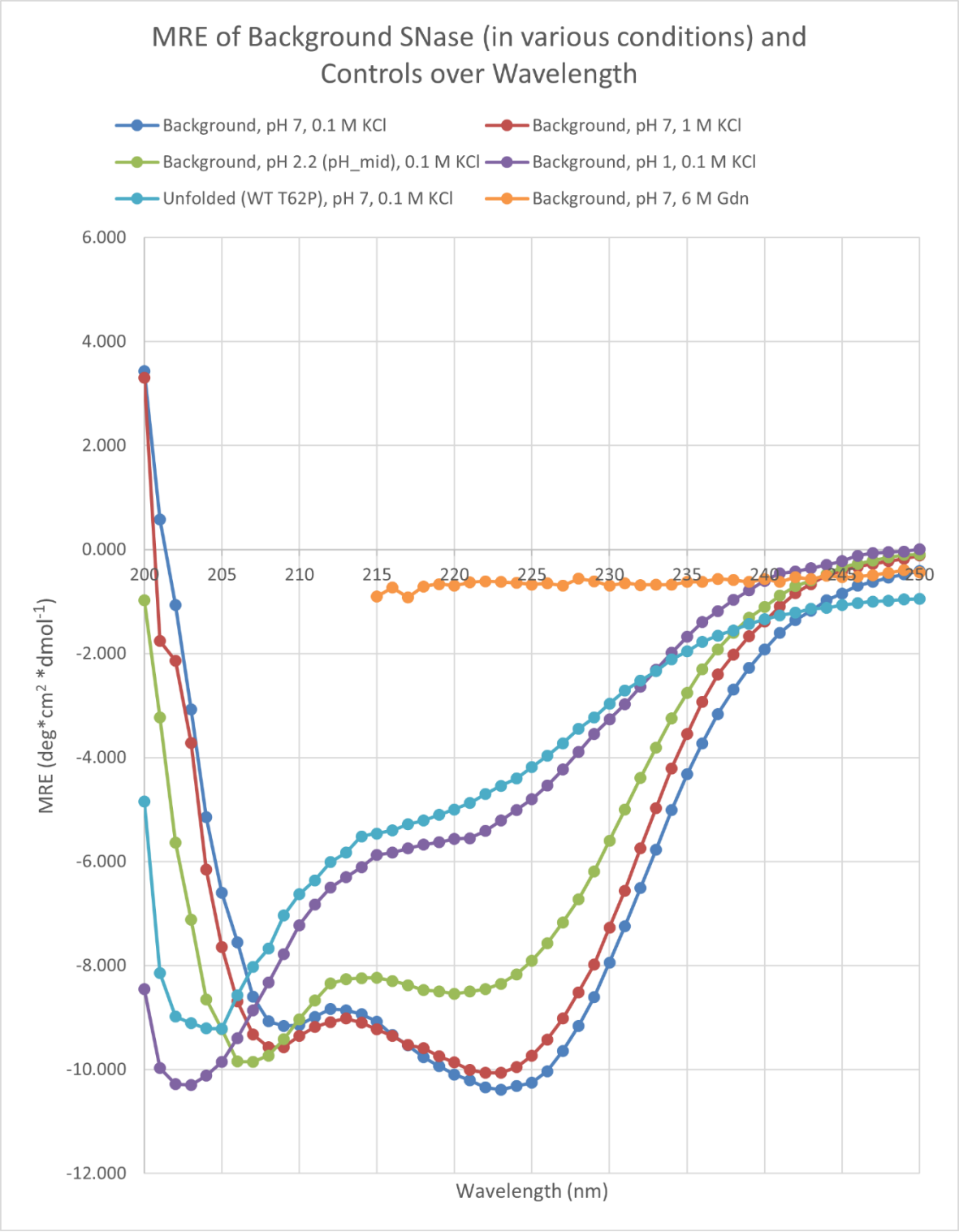
MRE of Background SNase (in various conditions) and Controls over Wavelength
Background, pH 7, 0.1 M KCl
Background, pH 7, 1 M KCl
Background, pH 2.2 (pH_mid), 0.1 M KCl
Background, pH 1, 0.1 M KCl
Unfolded (WT T62P), pH 7, 0.1 M KCl
Background, pH 7, 6 M Gdn
6.000
4.000
2.000
0.000
-2.000
-4.000
-6.000
-8.000
-10.000
-12.000
200
205
210
215
220
225
230
235
240
245
250
MRE (deg*cm2 *dmol-1)
Wavelength (nm)

Table 1:

Condition:	Protein:	MRE_{222}/MRE_{208}
pH 7, 0.1 M KCl	K63E-E67K	1.007
pH 7, 0.1 M KCl	Background	1.140
pH 7, 1.0 M KCl	K63E-E67K	1.234
pH 7, 1.0 M KCl	Background	1.052
pH 2.25, 0.1 M KCl	K63E-E67K	0.9500
pH 2.25, 0.1 M KCl	Background	0.8687
pH 1, 0.1 M KCl	K63E-E67K	0.5714
pH 1, 0.1 M KCl	Background	0.6500
pH 7, 0.1 M KCl	Unfolded	0.6121
pH 7, 6M GdnHCl	Denatured	N/A