<u>2.1</u>	<u>2.2</u>	<u>2.3</u>	<u>2.4</u>	<u>2.5</u>	<u>Total</u>
6	4	8	3	4	27

Problem 2: "As If It's Your Last" - BLACKPINK

There is no particular reason why this problem is titled "As If It's Your Last" beyond Anugrah and Michael's amusement. Animals and plants use lysozyme to kill invading Gram-positive bacteria. Lysozyme binds with the hexamer of N-acetylglucosamine oligosaccharide (NAG₆), hydrolyzing it into smaller NAG_x oligomers such as NAG₃, NAG₂, and NAG. The resulting products can remain bound to lysozyme and can inhibit enzymatic activity.

A solution of Lysozyme is titrated with a purified solution of NAG₃ while maintaining the reaction vessel at constant temperature. This is done by 25 10 μ L injections of 2.4 mM NAG₃ into a reaction vessel which contains 0.14 mM Lysozyme solution.



The heat absorbed from the reaction vessel per unit time is graphed (left). The heat per second data is manipulated to plot the net heat absorbed during each successive injection (right) versus the molar ratio of total NAG_3 concentration to total Lysozyme concentration (concentration of both bound and unbound forms) after that injection. The first 11 data points from the plot are also shown below.

Inj.	1	2	3	4	5	6	7	8	9	10	11
μJ	-1070	-1000	-950	-850	-680	-470	-285	-190	-120	-90	-70

<u>2.1</u> Using the titration data, determine the coefficient x in the general complex formation equation for NAG₃ and Lysozyme. Justify your answer thoroughly.

xNAG₃ + Lysozyme \rightleftharpoons Lysozyme-(NAG₃)_x

<u>2.</u> Compute approximately the ΔH_{rxn}° for the complexation reaction with NAG₃ <u>2.3</u> Compute the value of K_{f3} for the complexation reaction with NAG₃ <u>2.4</u> Compute ΔS_{rxn}° for the complexation reaction with NAG₃

If you could not determine x in question 2.1, then you can use x = 4 in 2.5 for full credit!

NAG complexes with Lysozyme in a 1:1 ratio. The equilibrium constant for the complexation of lysozyme with the monomer NAG is much smaller than that of NAG₃. In order to measure it, a small amount of NAG is placed into a 0.14 mM solution of Lysozyme, which is then titrated with the same method of injections. The apparent complex formation constant K_{app} between NAG₃ and Lysozyme in the presence of lysozyme is defined counting [Lysozyme] as if NAG had not partially complexed with it as follows:

$$K_{app} = \frac{[L - (NAG_3)_x]}{([L] + [L - NAG])[NAG_3]^x}$$

From this the following equation is obtained (where n is an integer)

$$\frac{K_{NAG_3}}{K_{app}} = n + K_{NAG}[NAG]$$

<u>2.5</u> Determine the value of *n* in the above equation.