es such as honey and saliva (Gajda, E., & Bugla-Płosko ska, G, 2014). The reason why using hen egg white is common is because 3.5% out of 11% of the total protein in this mixture is lysozyme, making it a good source of it. Considering the immense research done on hen egg white lysozyme, there is still a lot unknown between the differences and sim!ilarities between duck and hen as sources of lysozyme. These two are known to have similar structures, and when comparing their amino acid sequence, it is visible that they share some similarities in the active site (Figures 3 and 4). It is important to mention that for duck lysozyme three different isoforms are present independently from one another, and the one with higher similarity to hen lysozyme is duck egg-white lyso!zyme III (Jollès, J., et al., 1967). Furthermore, it has been studied that 1.2% of proteins in ducks are lysozyme (Hermann and Jollès, 1970).

/ .0 - %12 D42 F+ 7 C&%.#+ * 2 +82 #1G - 1 * ' 1#2 +82 61 * 2 & * , 2 , - ' H2 1 0 0 2 B6.$12 9<? sozyme using Cluster Omega protein sequence alignment. The top sequence is the corresponding to the hen and the bottom one is corresponding to the duck. The different symbols represent the simi!larity between the two sequences. The asterisk (*) represents a 100% match between the two. A colon (:) represents highly similar amino ac!ids. The period (.) is those amino acids that are slightly similar, and no symbol means no similarities. There are two regions highlighted with yellow that are the two amino acids used in the active site Glu 35 and Asp 52 in hen lysozyme. There is a conservation of the Glu 35 in duck, but no Asp 52, even though the Gly 52 present is slightly similar.

/ .0 - %12 I42 (6 1 2 #$% - ' $ - %12 +82 61 * 2 9<#+=< 7 12 , 1C. ' $1 , 2 . * 2 0%11 * 2 # - ? C1%. 7 C+#1 , 2 $+2 $612 #$% - ' $ - %12 +82 , - ' H2 9<#+=< 7 12 . * 2 +%& * 0142 Us!ing the Dali server for 3D global protein comparison, the structure of the lysozyme from the two sources can be aligned and allows for vi!sualization of homology between these two. The areas colored blue are the regions where the structure is conserved among the two.

In this experiment, the aim is to extract lysozyme from duck egg whites and compare it to the lysozyme from a stock solution of hen lyso!zyme. To do this, f rst, the duck egg white will be isolated and purif ed using ion exchange chromatography, followed by purif cation and enzymatic ac!tivity check with gel electrophoresis and protein assays. After the best frac!tions of pure lysozyme are identif ed these will undergo a process of dialy!sis and concentration to prepare them for enzyme kinetics. In this step, the enzymes from both sources will be analyzed creating two plots: Michae!lis-Menten and Lineweaver-Burk plots, to accurately depict their catalytic properties. Based on the percentage content of lysozyme for each source, it is hypothesized that the hen and duck will differ. In particular, the hen egg will have a higher rate of reaction when presented with the same bacteria.

J1# - 9$#

Successful purif cation of lysozyme from duck egg white

To use the lysozyme from the duck egg white, we f rst had to ex!tract it and purify it. For this, ion exchange chromatography was used, followed by spectrophotometer analysis using bacteria. For detailed pro!tocol refer to the methods section. The egg white was separated from oth!er components of the egg, buffered, and run through the column with a negatively charged resin. The fractions obtained were then analyzed for an enzymatic activity to test if lysozyme was present. This was possible because of the hydrolytic activity that the lysozyme has with the bacte!ria, so by analyzing the absorbance at 450 nm of the latter one we can determine if they are being destroyed. This means if the bacteria are be!ing cleaved, the absorbance decreases, as a result of more light going through and reaching the detector, resulting in a steeper slope. After ob!

Firstly, the positive control, lane 2, showed the expected size for lysozyme at approximately 15 kDa. The fow through and the washes also showed multiple bands as expected, with the fow through having too many components from the egg white. The intensity and shape of the fow through bands is also an indicator that too much protein sample was load! ed. For the fow through, it is expected to fnd enzymatic activity because

/.0-%12M425.'6&19.#N51*$1*2C9+$28+%2;<#+=<7128%+72,-'H2100 2B6.$12 8+%28%&'$.+*234 The absorbance was measured at 450.4 nm. Each dot represents a different reading with the same enzyme concentration of 0.038 mg/mL and different substrate concentrations.

/.0-%12O42;.*1B1&:1%?P-%H2C9+$28+%2;<#+=<7128%+72,-'H2100 2B6.$12 8+%28%&'$.+*2342The reciprocal of the initial velocity is graphed out as a function of the reciprocal of substrate concentration. A trendline was added to the data set, with the slope depicted in the top right corner with an equation of y = 1.9686x + 3331.1, followed by the r-squared value of 0.0093.

Figures 6 and 7, show an evident pattern that there are not enough data points obtained to make an accurate decision of enzymatic kinetics for this fraction. From the graph, the slope is y =1.9689x + 3331.1, and the R-squared = 0.0093, which denotes a high variance in the data set. The same procedure was followed for fraction 2+3, and the results are shown in fgures 8, 9, and 10.

/.0-%12Q425.'6&19.#N51*$1*2C9+$28+%2;<#+=<7128%+72,-'H2100 2B6.$12 8+%28%&'$.+*2@RD4 The absorbance was measured at 450.4 nm. Similar! ly, each blue dot is one trial with constant [E] = 0.039 mg/mL. The curve shows an almost parabola.

Figure 8 shows the Michaelis-Menten plot for the duck Lysozyme, when [E] = 0.038 mg/mL, [S1] = 0.15mg/mL, and [S2] = 0.6 mg/mL. Be! cause we see the rapid increase and then a sudden decrease, similar to a parabola, it is evidence to say the enzyme was too saturated. For this reason, we excluded the last two data points, depicted in fgure 9. Values smaller for bacteria concentration should have been measured but due to the lack of the enzyme, this part couldn't be followed-up with more trials.

/.0-%12S42TC,&$1,25.'6&19.#N51*$1*2C9+$28+%2;<#+=<7128%+72,-'H21002 B6.$128+%28%&'$.+*2@RD4 The absorbance was measured at 450.4 nm. The three blue dots represent three different trials with different substrate con!

/.0-%123@42;.*1B1&:1%?P-%H2C9+$28+%2;<#+=<7128%+7261*2#$+'H42The trendline is present with the slope equation, y = 9.2917x + 31.587, fol

Proctor, V. A., & Cunningham, F. E. The chemistry of lysozyme and