

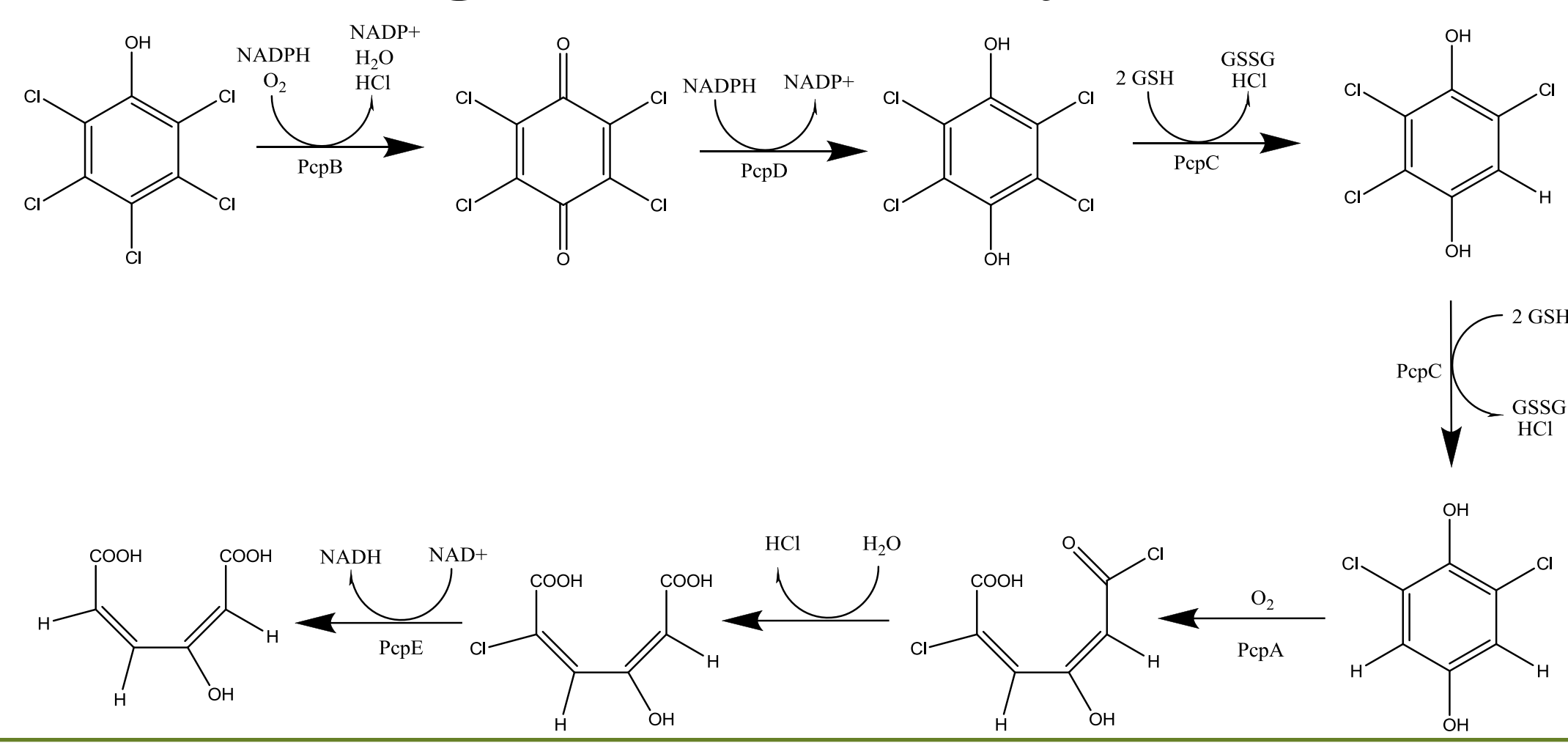
1

Introduction

Pentachlorophenol (PCP) is a fungicide that was primarily used since the 1920's to preserve pressure treated lumber from rotting, but was banned in 1987 due to various health hazards associated with PCP leaching into the nearby soil and water systems and also because PCP does not naturally degrade very easy. One of the ways PCP naturally degrades is through a series of enzymatic proteins present in the organism *Sphingobium chlorophenolicum*. The Kang Lab has set out to crystallize the series of proteins found in *Sphingobium chlorophenolicum* then solve their structure through X-ray crystallography, in which a beam of X-rays are shot at the protein crystal and diffracts into multiple locations which is called a diffraction pattern and is specific to the individual protein. The diffraction pattern can be used to give an electron density map and can then be used to place individual atoms in the protein and determine the structure.

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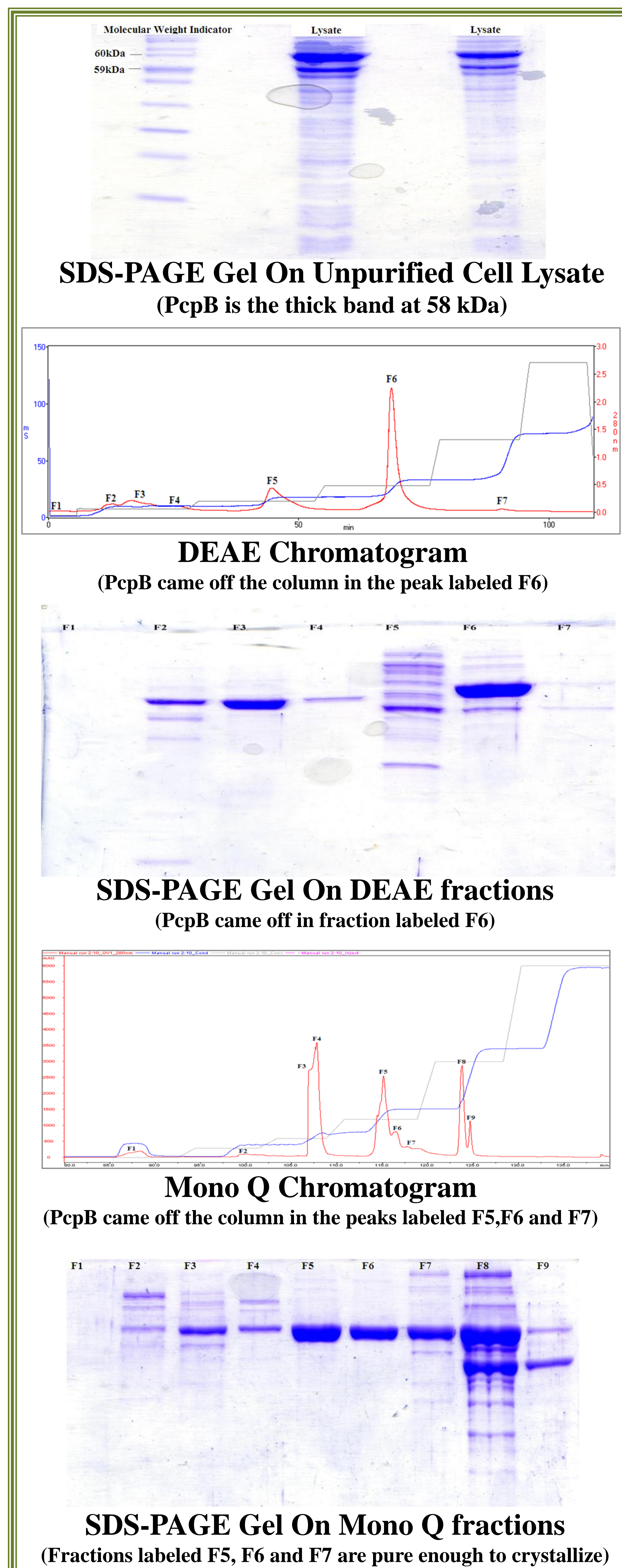
Biodegradation Pathway of PCP



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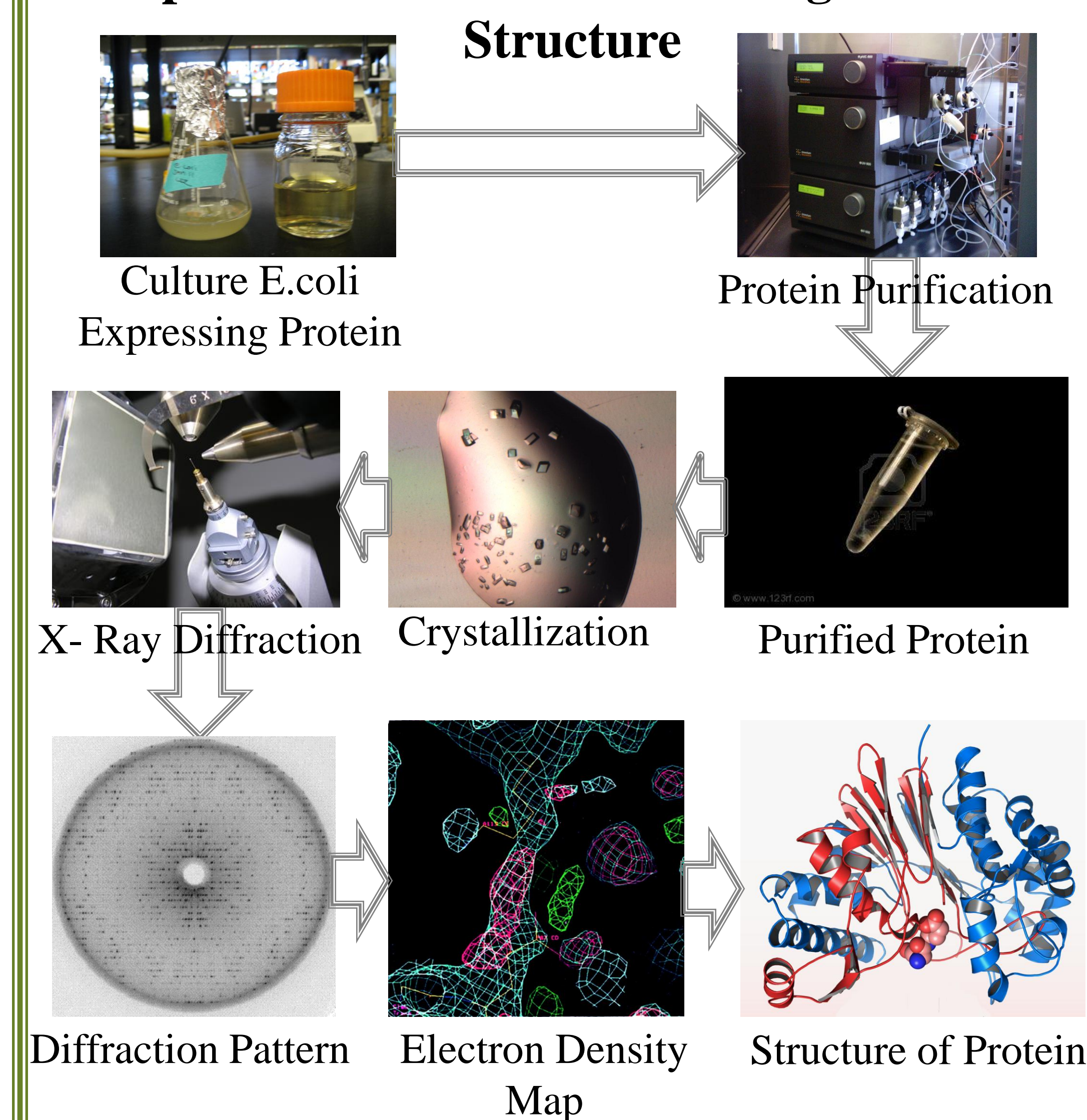
Research Plan

In an effort to crystallize PcpB, transfected *E. coli* cells containing the PcpB construct must be cultured and then lysate the cell membranes, leaving the *E. coli* protein in solution and the insoluble cell membranes to be separated by centrifuge. The protein must then be purified in order to separate it from other *E. coli* proteins. Protein purification can be accomplished by use of a variety liquid chromatography and HPLC columns such as Phenyl Sepharose, DEAE and Mono-Q. After every purification step an SDS-Page gel is ran on the fractions to determine where the PcpB came off and if further purification is necessary. Once the PcpB is pure enough, the concentration can be determined by use of a BCA assay and the PcpB can be further concentrated to optimum concentration for crystallization. The protein is crystallized on a small scale by mixing the protein sample with various crystal screen reagents supplied by Hampton Research and allowed to crystallize in a cold room. Any protein crystals that form can be removed from the solution and loaded into the X-ray diffractometer to solve the structure.



4

Steps involved with Determining a Protein Structure



5

Conclusion

PcpB was successfully purified and crystallized using a crystal screen reagent consisting of 0.2M solution of zinc acetate dihydrate containing 20% w/v polyethylene glycol. The crystals that were formed were not of ideal shape for X-ray diffraction. Further modification of the crystal screen formula may be necessary if the current crystals don't produce an adequate diffraction pattern.

6

Future Work

The crystals of PcpB can be diffracted using X-ray crystallography, which will yield a electron density map of PcpB. The electron density map along with the known sequence of PcpB can be used together to solve the structure of PcpB.

Acknowledgements



This work was supported by the National Science Foundation's Research Experience For Undergraduates under grant number #CHE0851502