

Alkaline Phosphatase in the oligotrophic ocean: A $\delta^{18}\text{O}$ analysis of microbial activity

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SENIOR CHEMISTRY THESIS, SPRING 2016

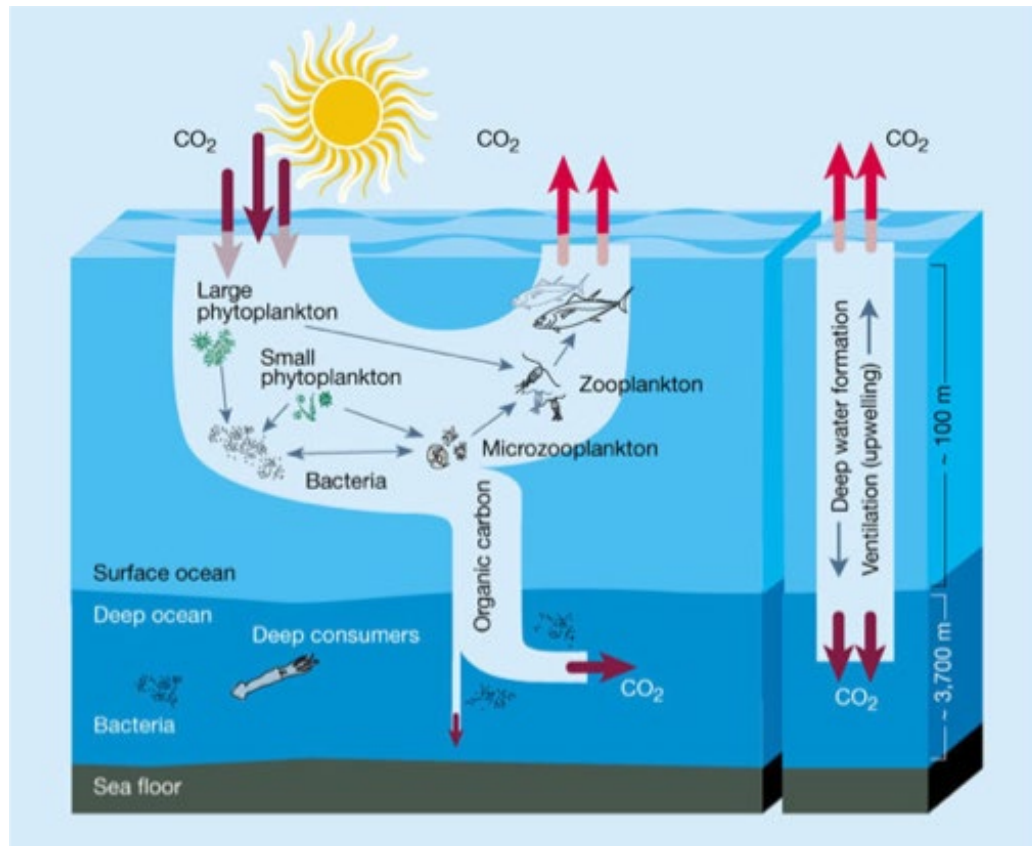
ADVISOR: ALBERT COLMAN, GEOPHYSICAL SCIENCES

Overview

- I. Background
- II. Experimental Overview
- III. Analytical Methods
- IV. Results
- V. Future Directions

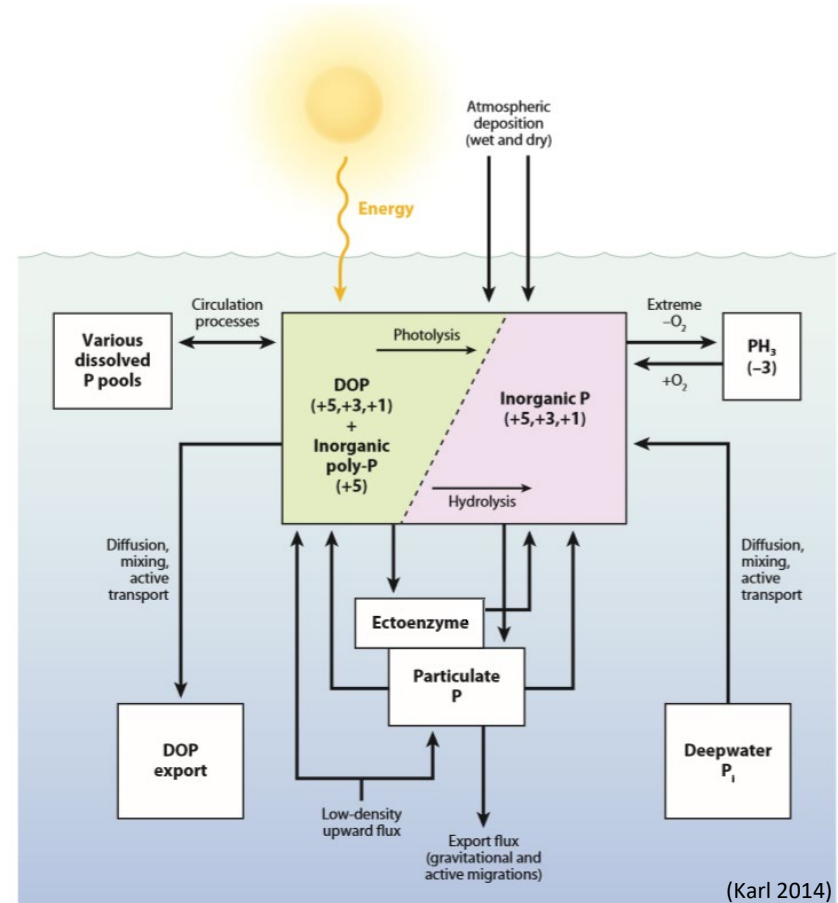
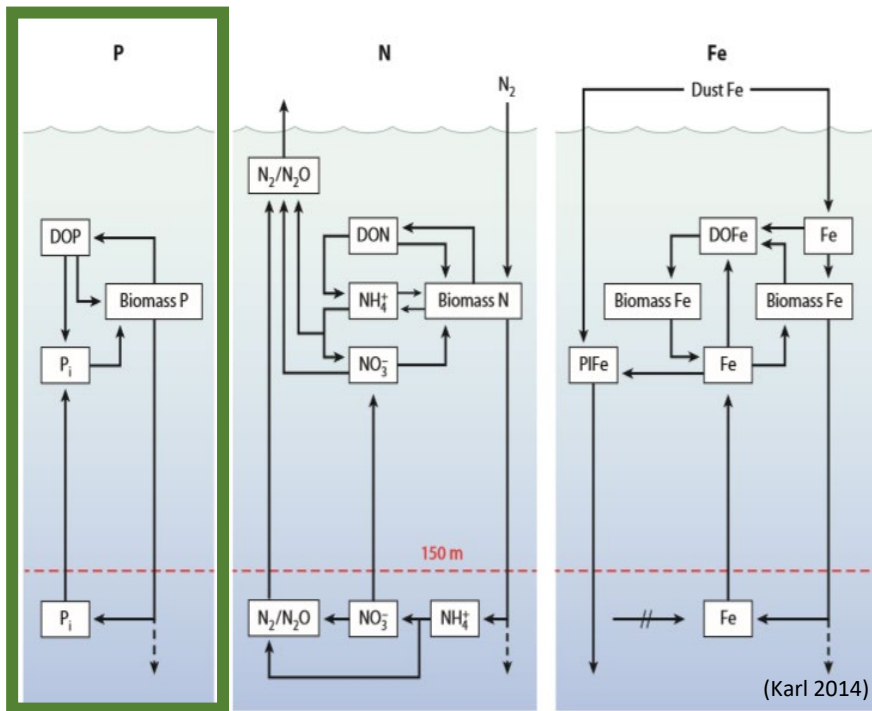
I. Background

Primary Producers



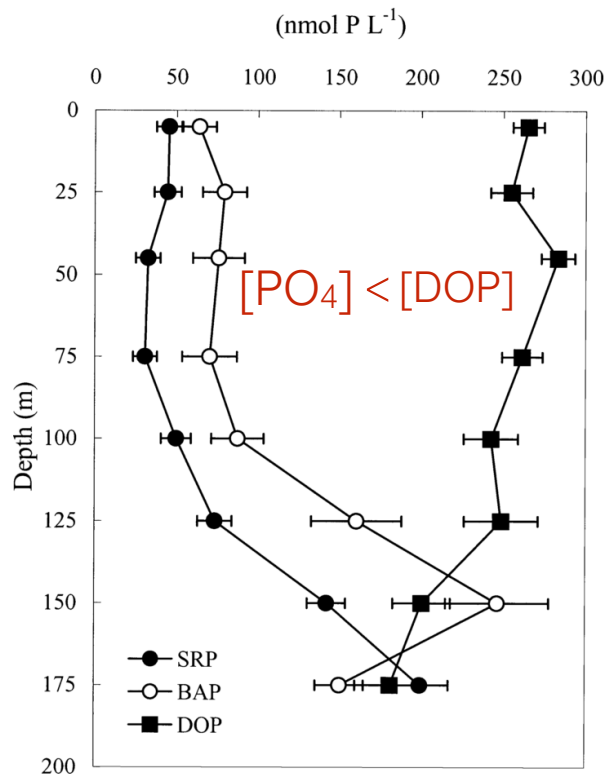
(Chisholm, 2000)

The Nutrient Cycle



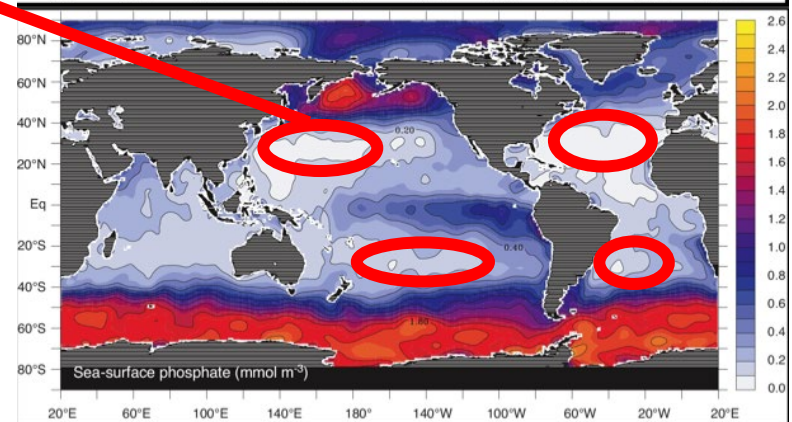
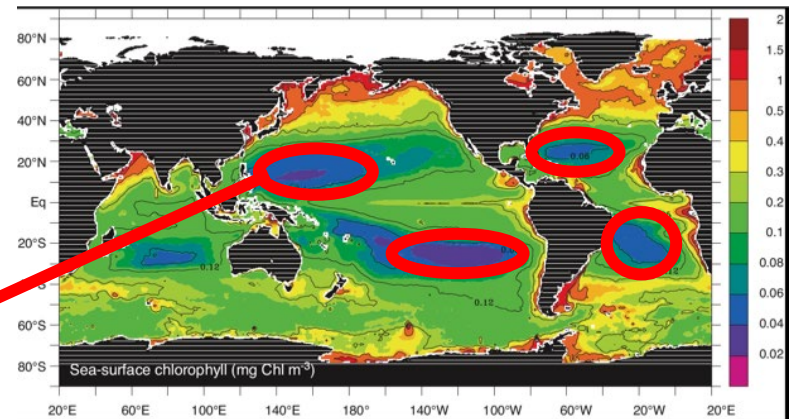
- Redfield Ratio – 106:16:1 (C:N:P)

Drivers of Primary Production



(Bjorkman and Karl, 2003)

Oligotrophic
Regions



(Sarmiento and Gruber, 2006)

Pi Cycling by Bacteria in Open Ocean

- Phosphate Pools

- Dissolved “Organic” Phosphate

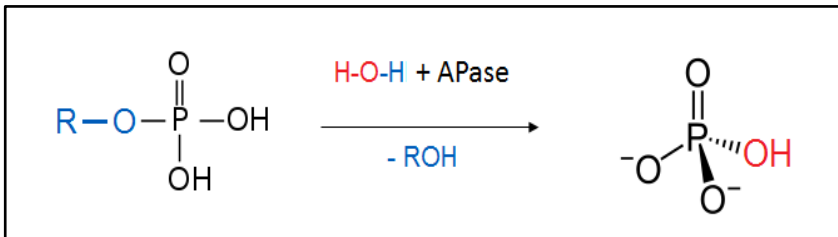
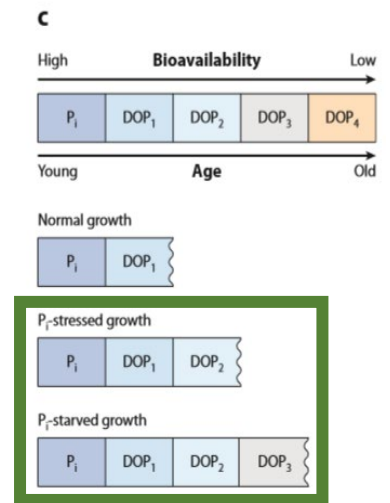
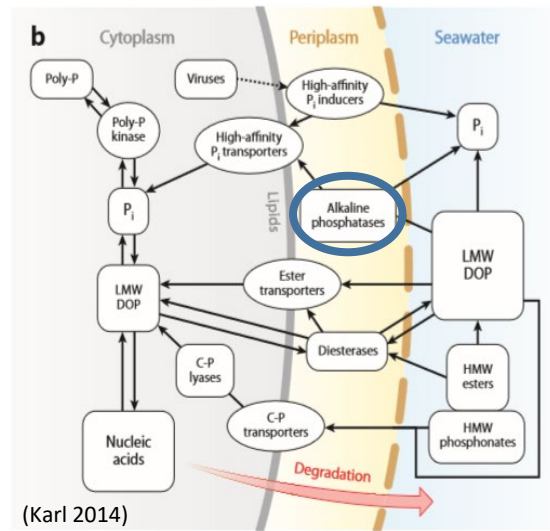
- “DOP”

- $(RO)_n P(OH)_{n-4}$

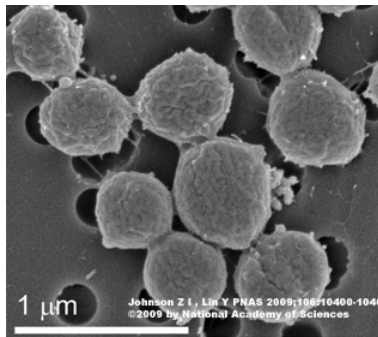
- “Inorganic” Phosphate

- “Pi”

- $H_2PO_4^-$, HPO_4^{2-} , PO_4^{3-}



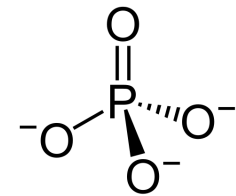
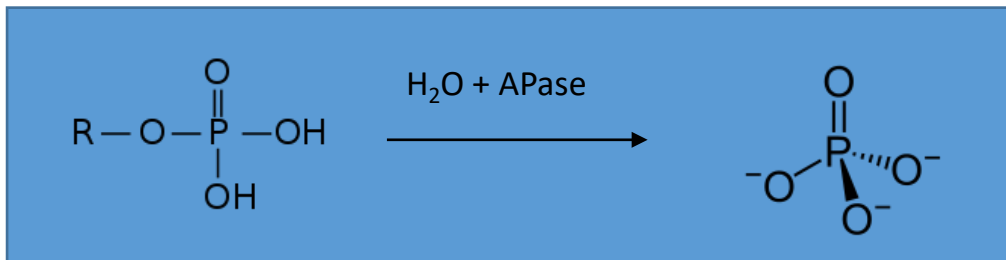
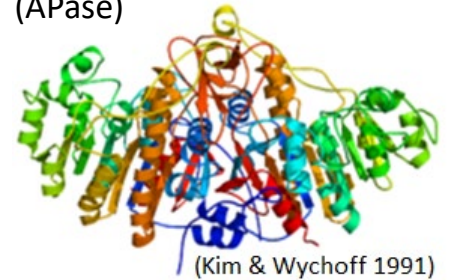
Moving from the Macro to Micro Level



Prochlorococcus

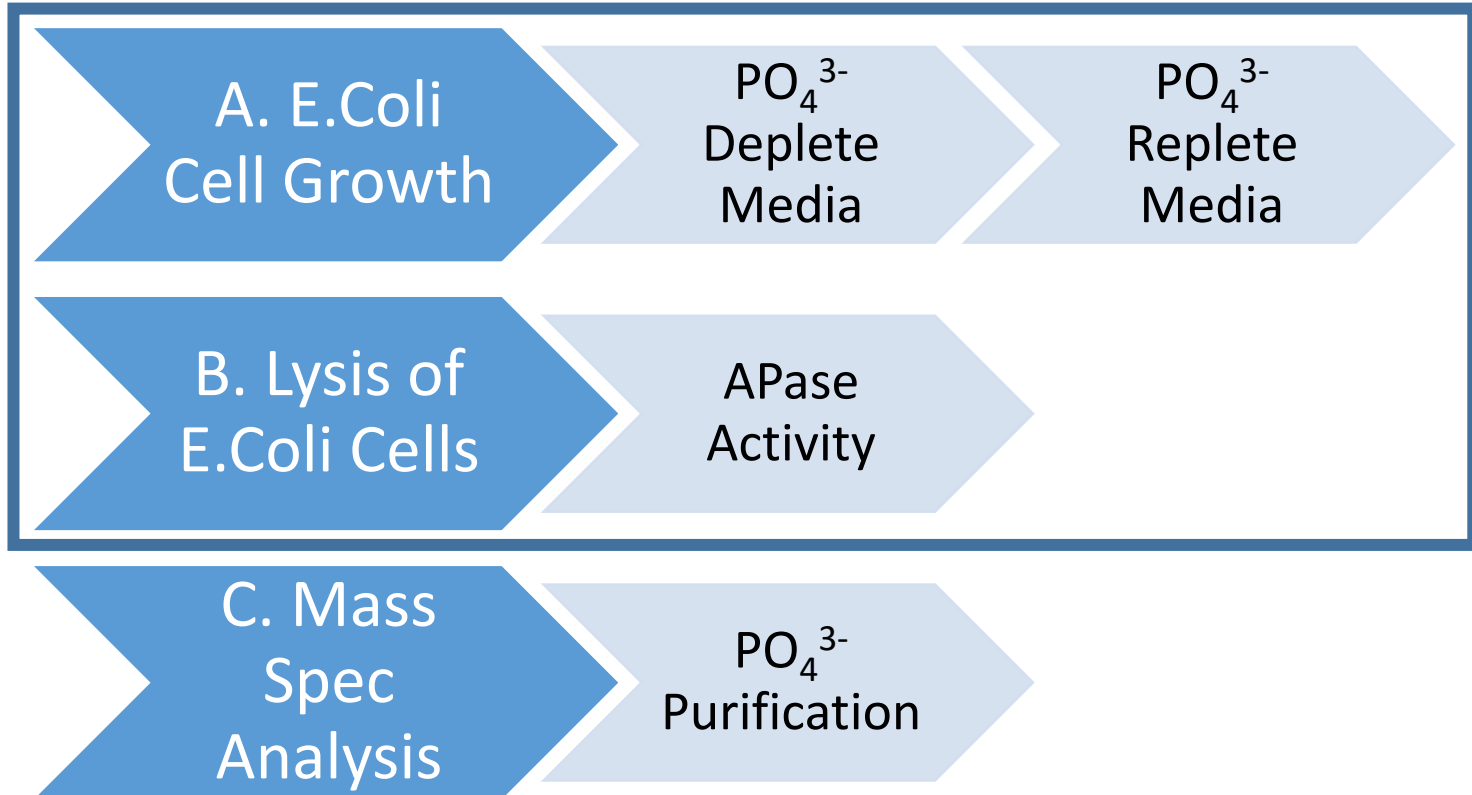


Alkaline Phosphatase
(APase)



II. Experimental Overview

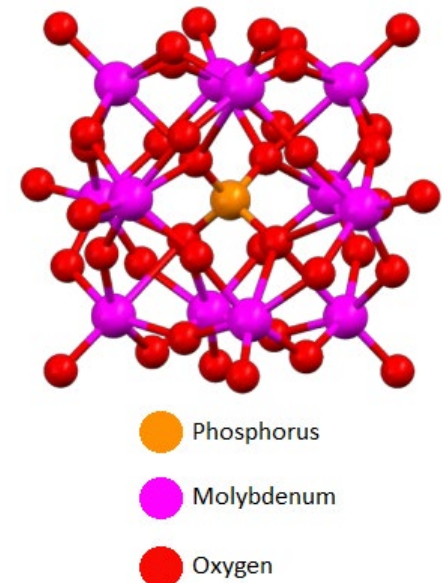
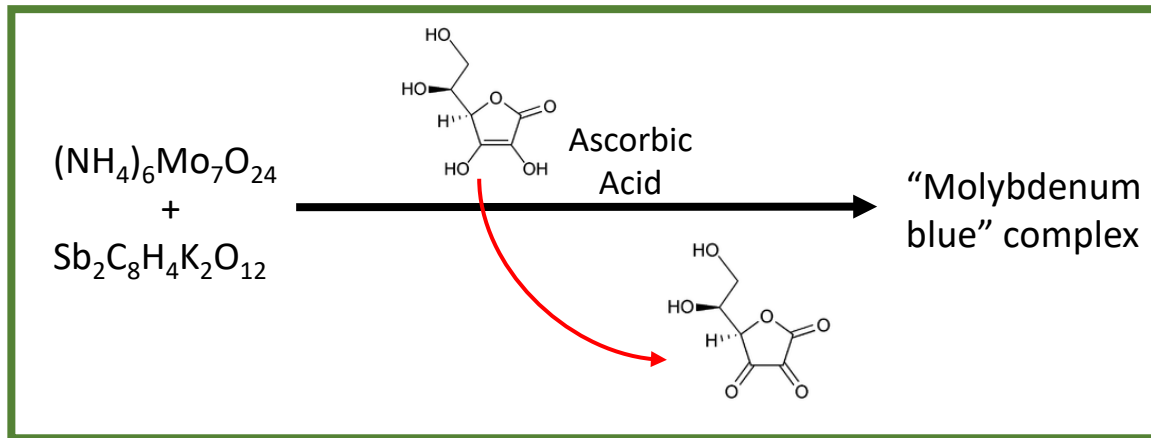
Experimental Overview



III. Analytical Methods

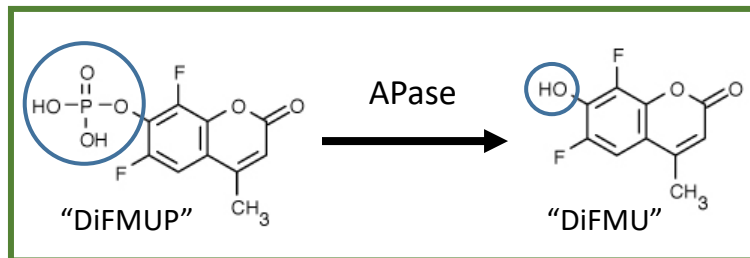
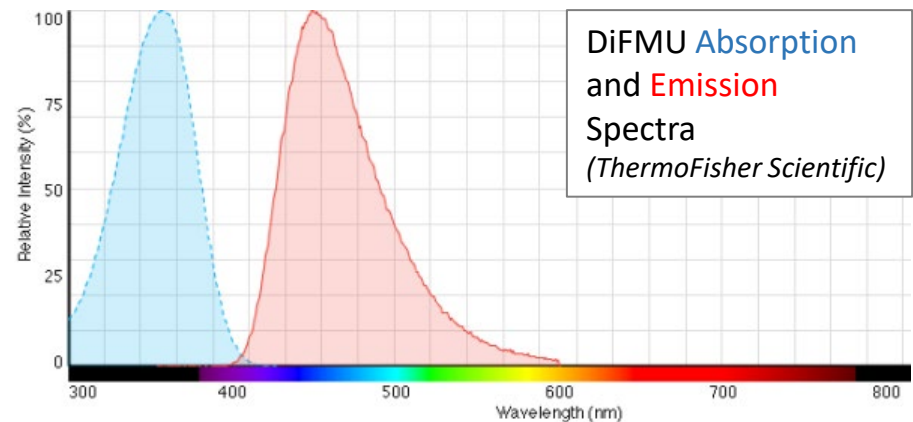
Spectroscopic Quantification of Pi

- Method widely used in natural samples, As and Si complicate complex
- Measures “Soluble Reactive Phosphate” (SRP)
 - PO_4^{3-} & HPO_4^{2-} dominate



Spectroscopic Tracing of APase Activity

- Cleavage of H_2PO_4^- from the DOP compound "DiFMUP" produces the fluorescent "DiFMU" product



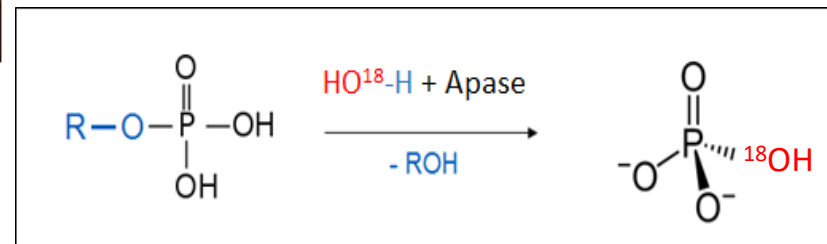
Stable Isotope Labeling & Mass Spec.

Legend for stable isotope labeling (Atomic Number):

- 7: Blue
- 6: Dark Blue
- 5: Light Blue
- 4: Cyan
- 3: Yellow-Cyan
- 2: Yellow
- 1: Orange
- 0: Red

Periodic table showing elements color-coded by their stable isotope labeling status. Elements are color-coded according to the legend on the right.

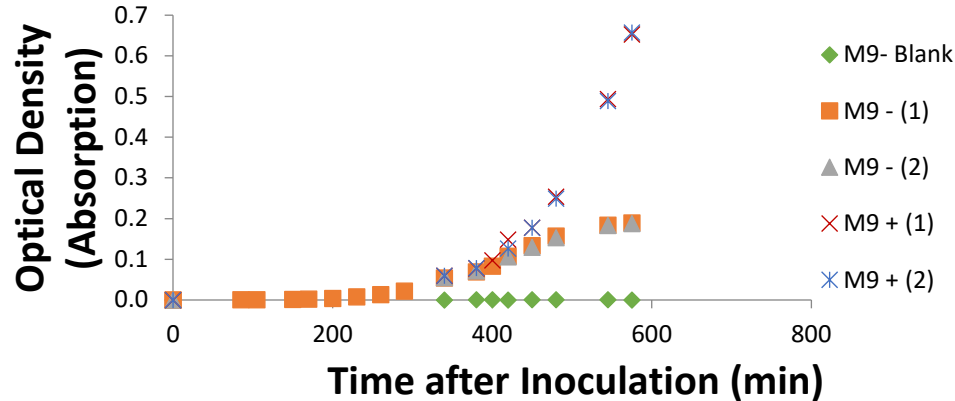
Oxygen Isotopes	Natural Abundance
^{16}O	99.76%
^{17}O	0.04%
^{18}O	0.20%



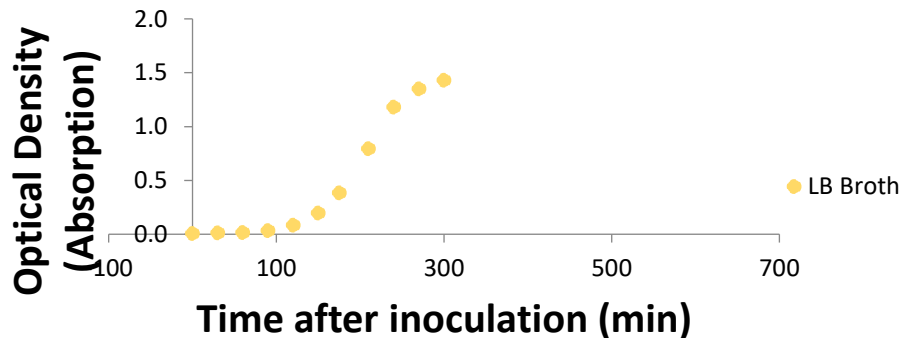
IV. Results

A. Cell Growth

E.Coli Growth in M9 +/- Media

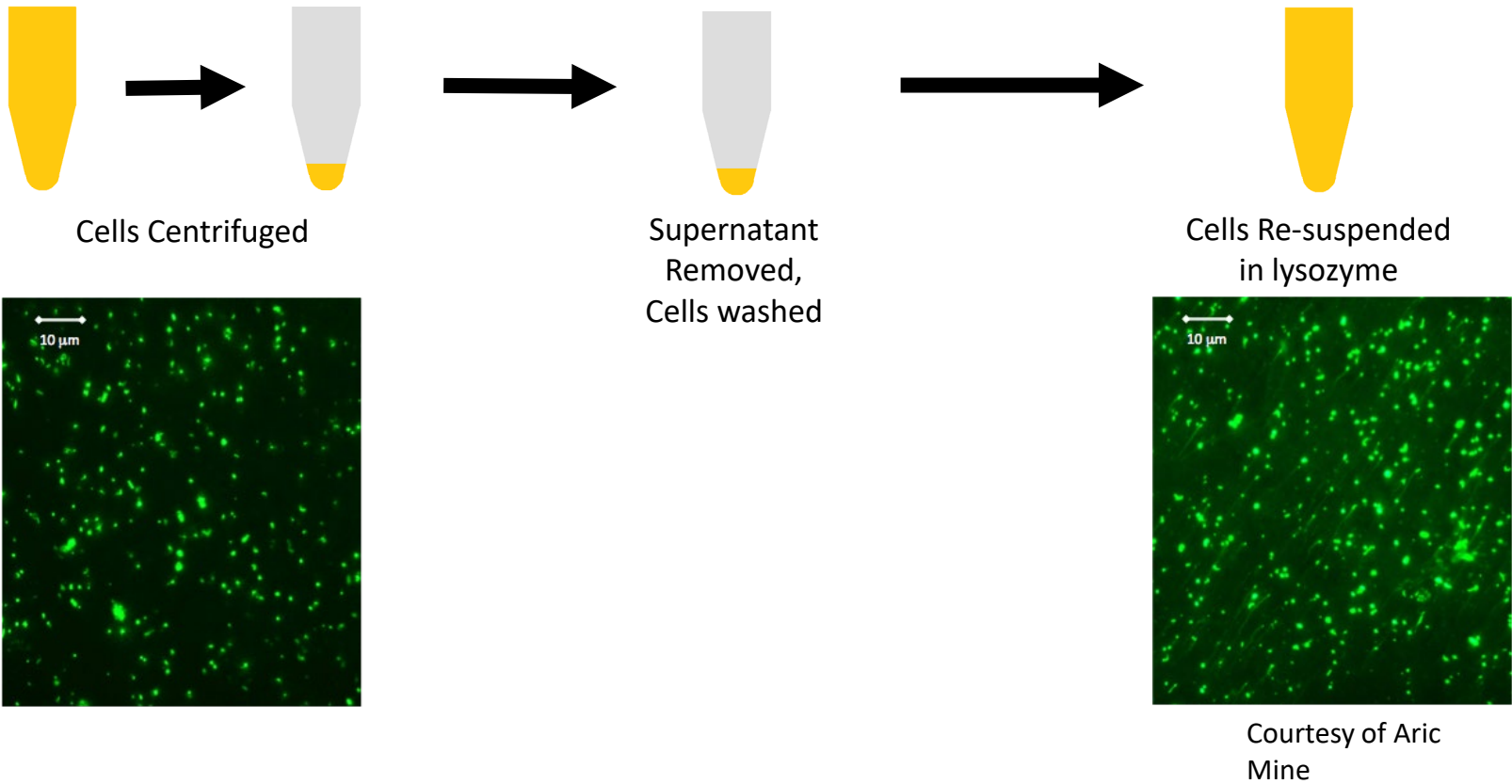


E.Coli Growth in LB Broth



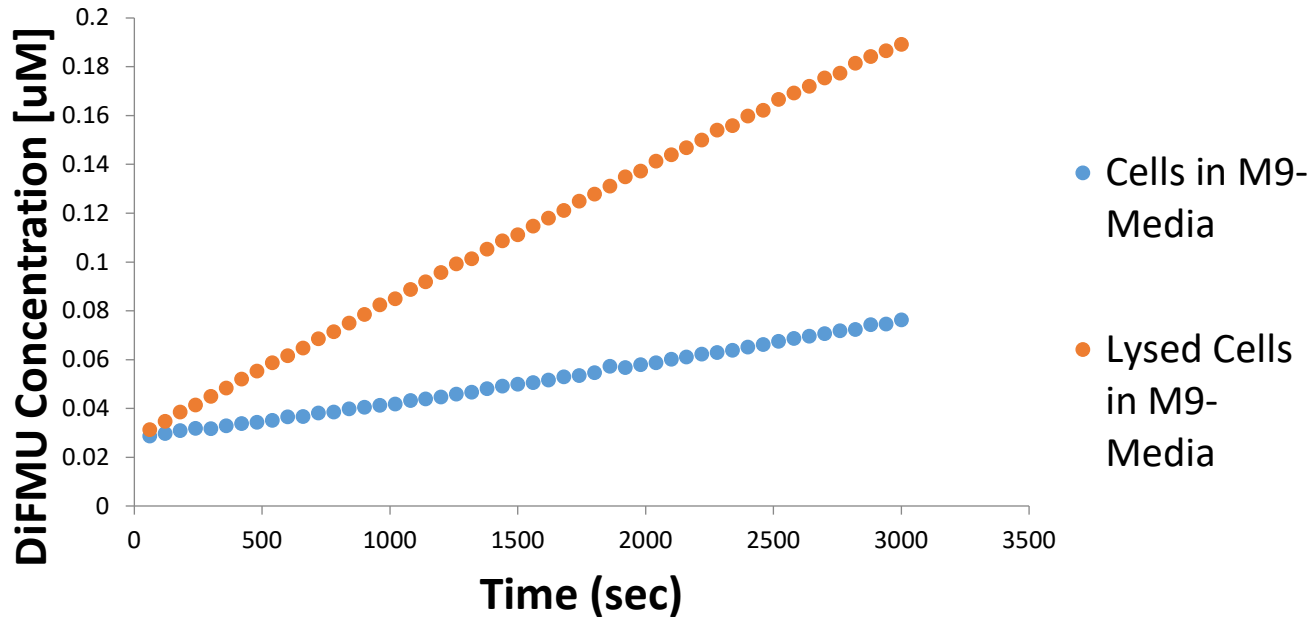
- Growth Media
 - M9+ (Pi Replete)
 - 700mM Pi
 - M9- (Pi Deplete)
 - 5.8mM GYP
 - 5.8uM Pi
- M9- selects for APase expression
 - DOP dominates as source for P
- *E.Coli* K-12 MG1665

B. Cell Lysis



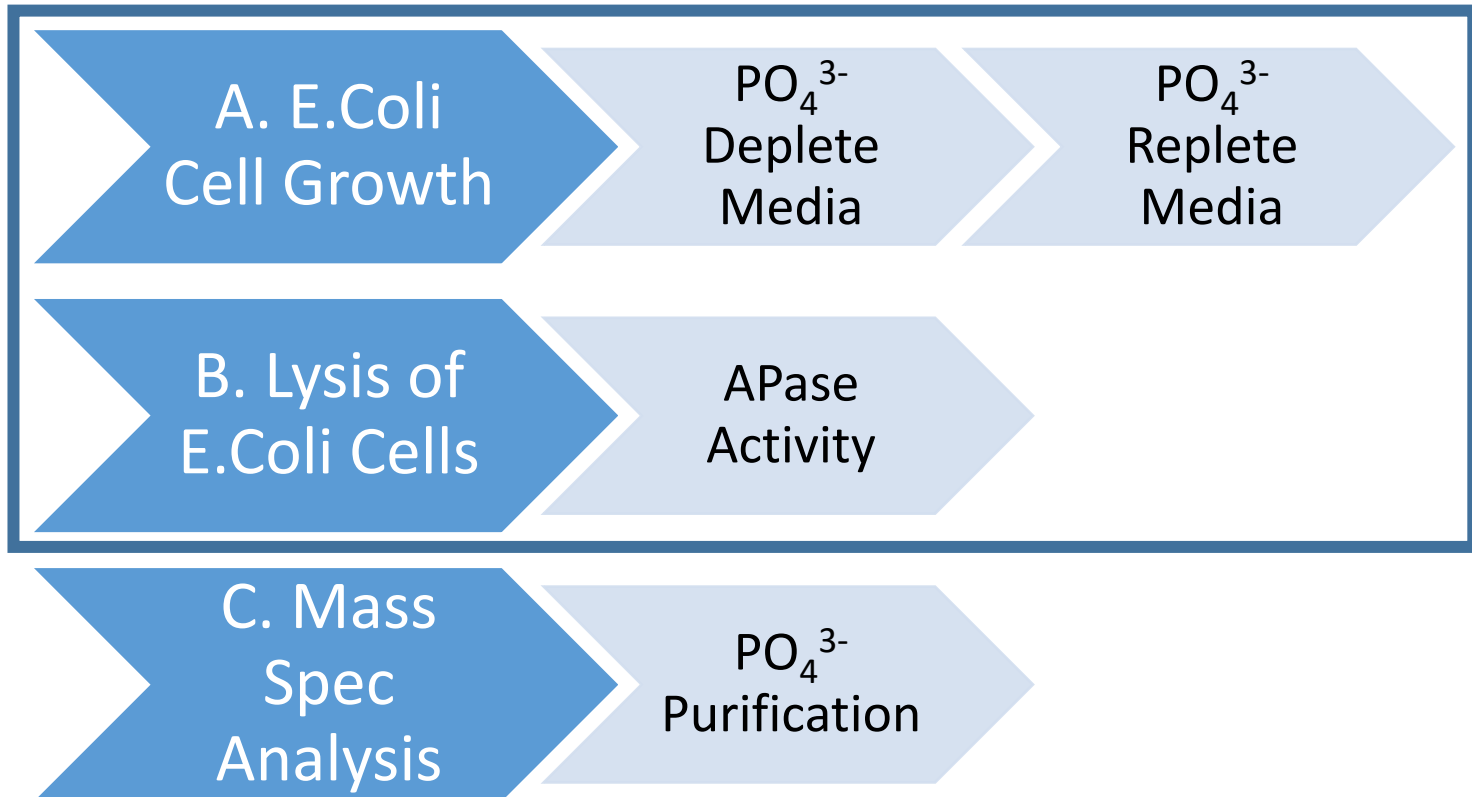
C. Cell Lysis and APase Activity

Lysed and Unlysed DiFMUP for P-limited



V. Future Directions

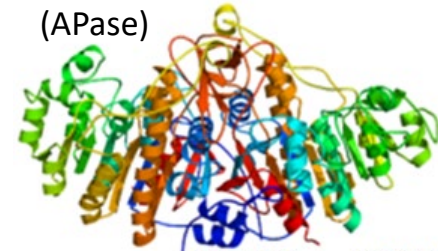
V. Future Direction



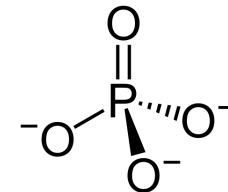
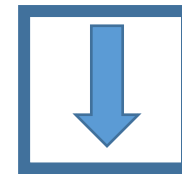
Future Direction: Pi isolation from M9 media

- Generalized Method
 - MagIC
 - Magnesium Induced Co-precipitation
 - Remove salts from medium
 - Cation/Anion Exchange
 - Remove salts and lysozyme from medium
 - Ag_3PO_4 Precipitation of Pi
 - $\delta^{18}\text{O}$ Measurements on MS

Alkaline Phosphatase
(APase)

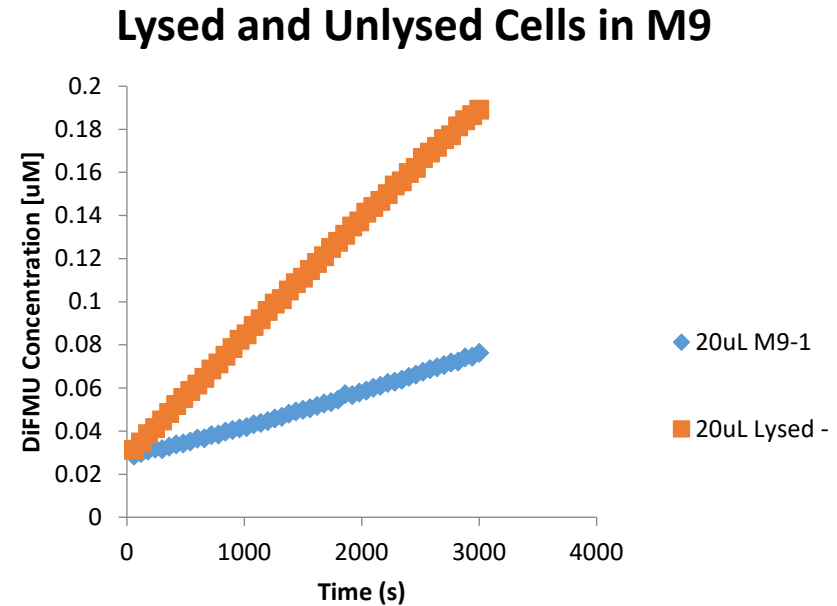
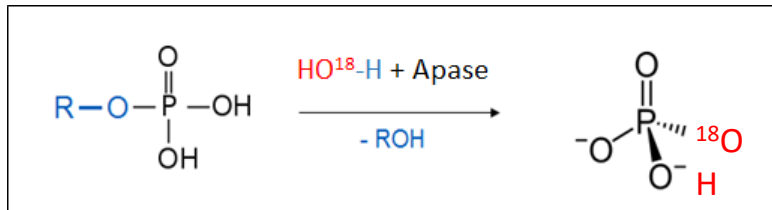


(Kim & Wychoff 1991)



Future Direction: $\delta^{18}\text{O}$ Measurements

- Isotope fractionation during growth
 - Kinetic fractionation of $\delta^{18}\text{O}$ during uptake or release of Pi
- Isotopic labeling of APase activity
 - Using $\delta^{18}\text{O}$ to track the cleavage of Pi from DOP compounds POST lysis



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