

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

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SENIOR CHEMISTRY THESIS, SPRING 2016

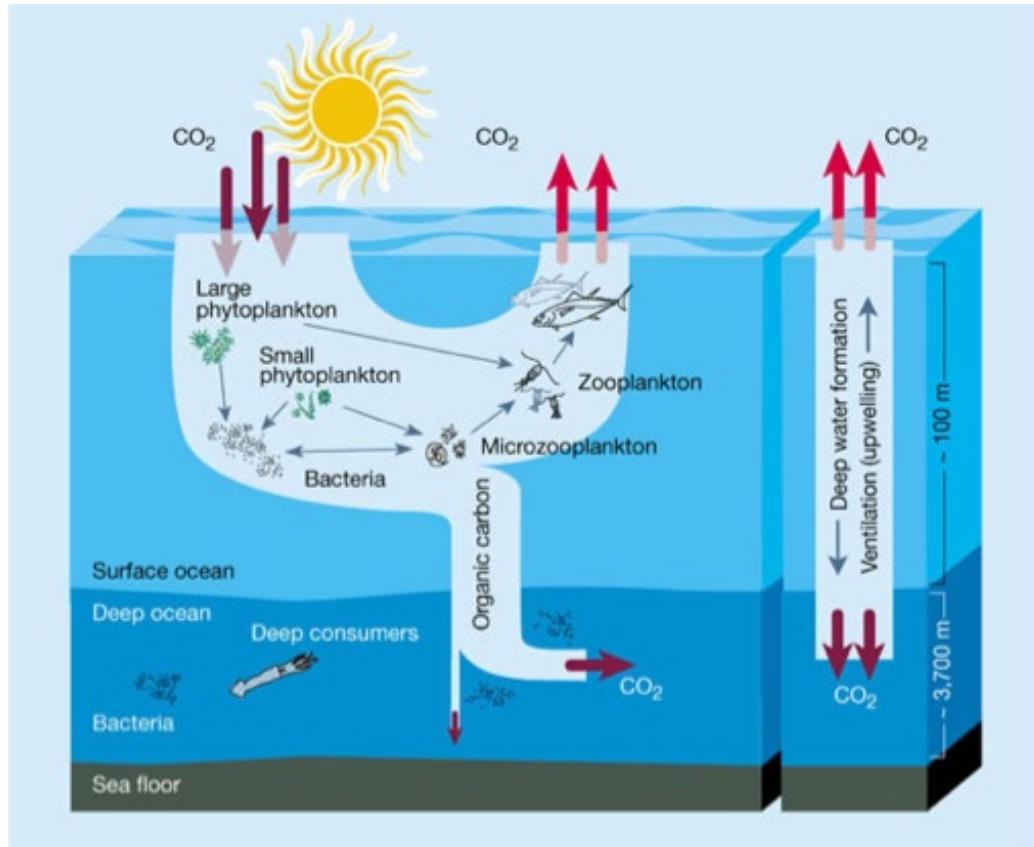
ADVISOR: ALBERT COLMAN, GEOPHYSICAL SCIENCES

Overview

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

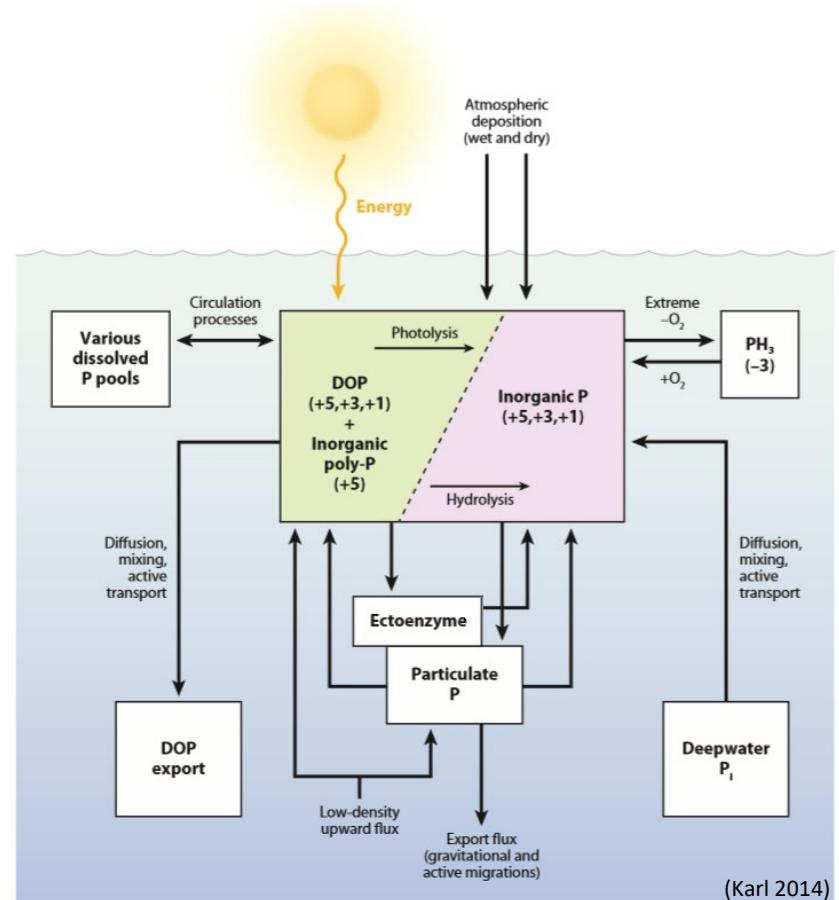
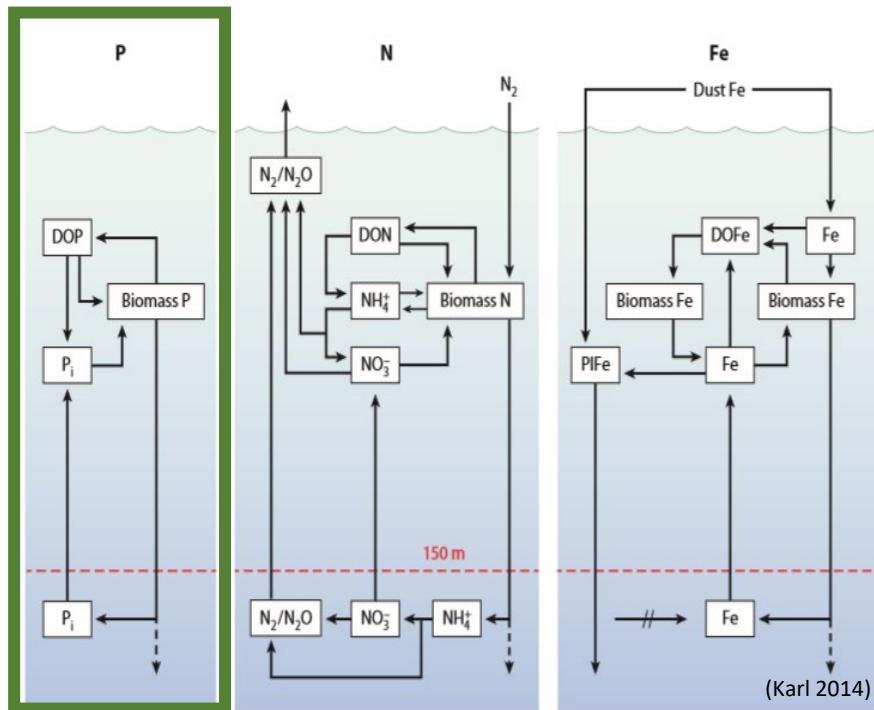
I. Background

Primary Producers



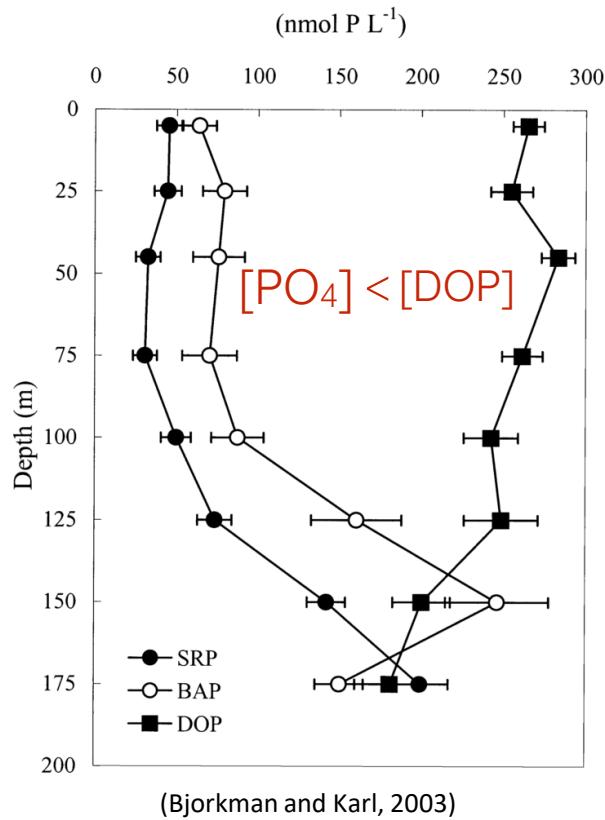
(Chisholm, 2000)

The Nutrient Cycle

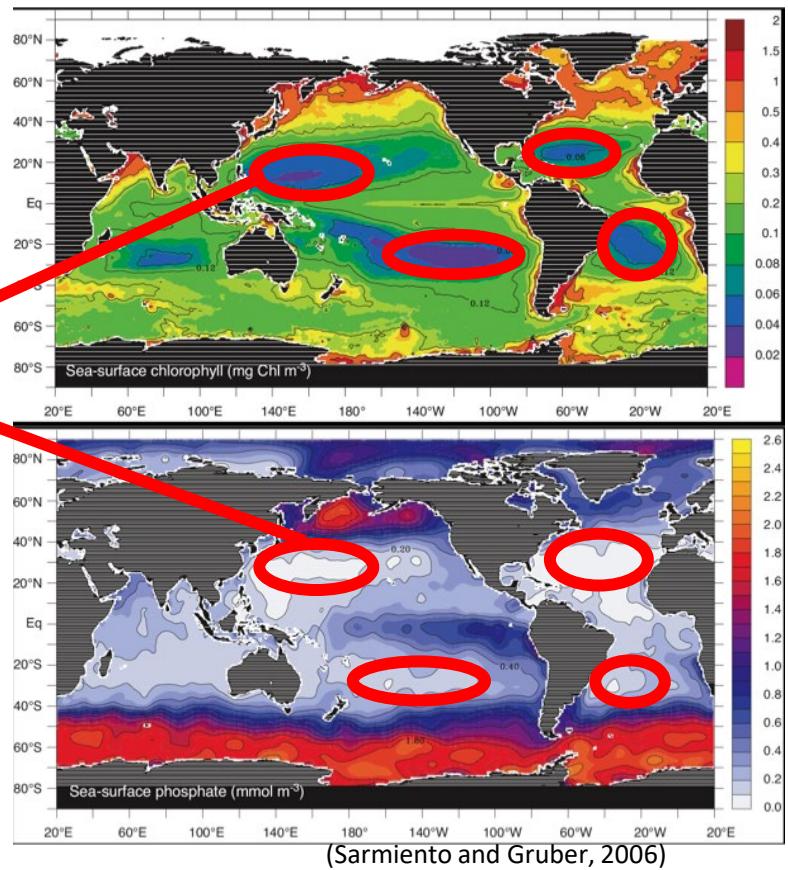


- Redfield Ratio – 106:16:1 (C:N:P)

Drivers of Primary Production



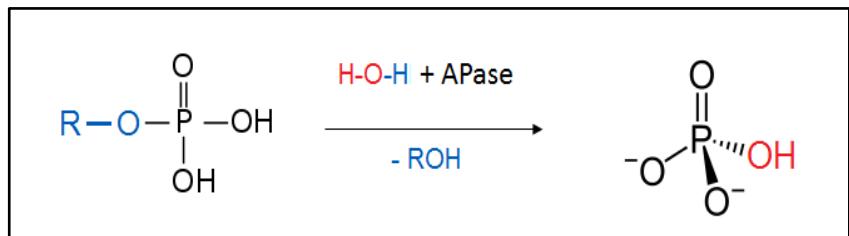
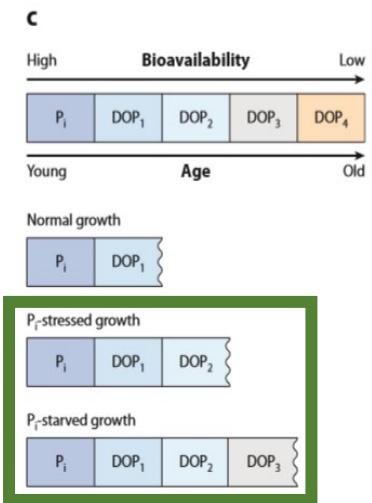
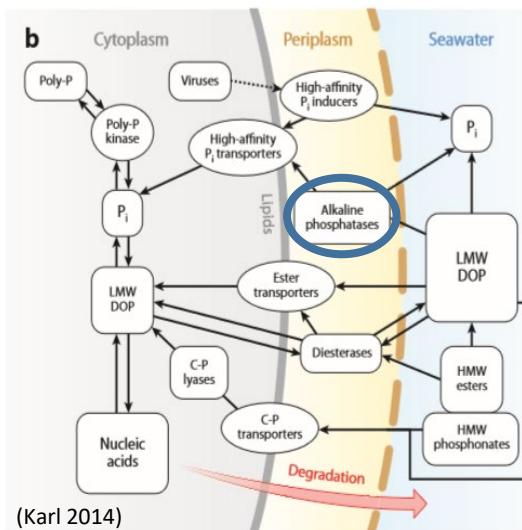
Oligotrophic
Regions



Pi Cycling by Bacteria in Open Ocean

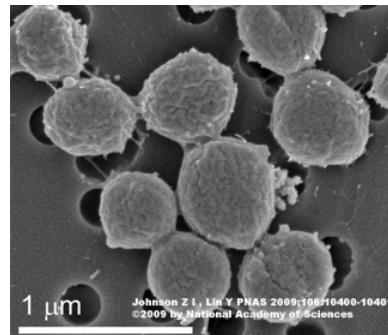
- Phosphate Pools

- Dissolved “Organic” Phosphate
 - “DOP”
 - $(RO)_n P(OH)_{n-4}$
- “Inorganic” Phosphate
 - “Pi”
 - $H_2PO_4^-$, HPO_4^{2-} , PO_4^{3-}



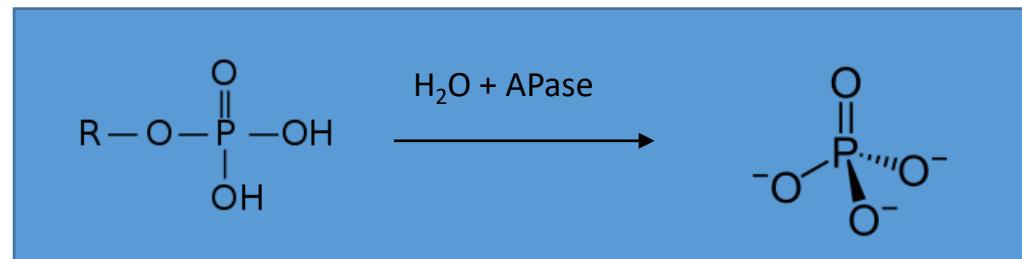
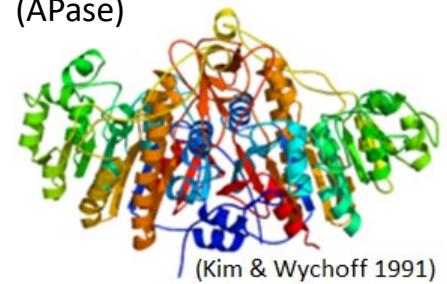
Background

Moving from the Macro to Micro Level

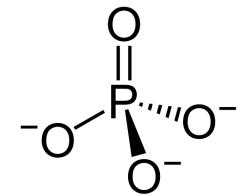


Prochlorococcus

Alkaline Phosphatase (APase)

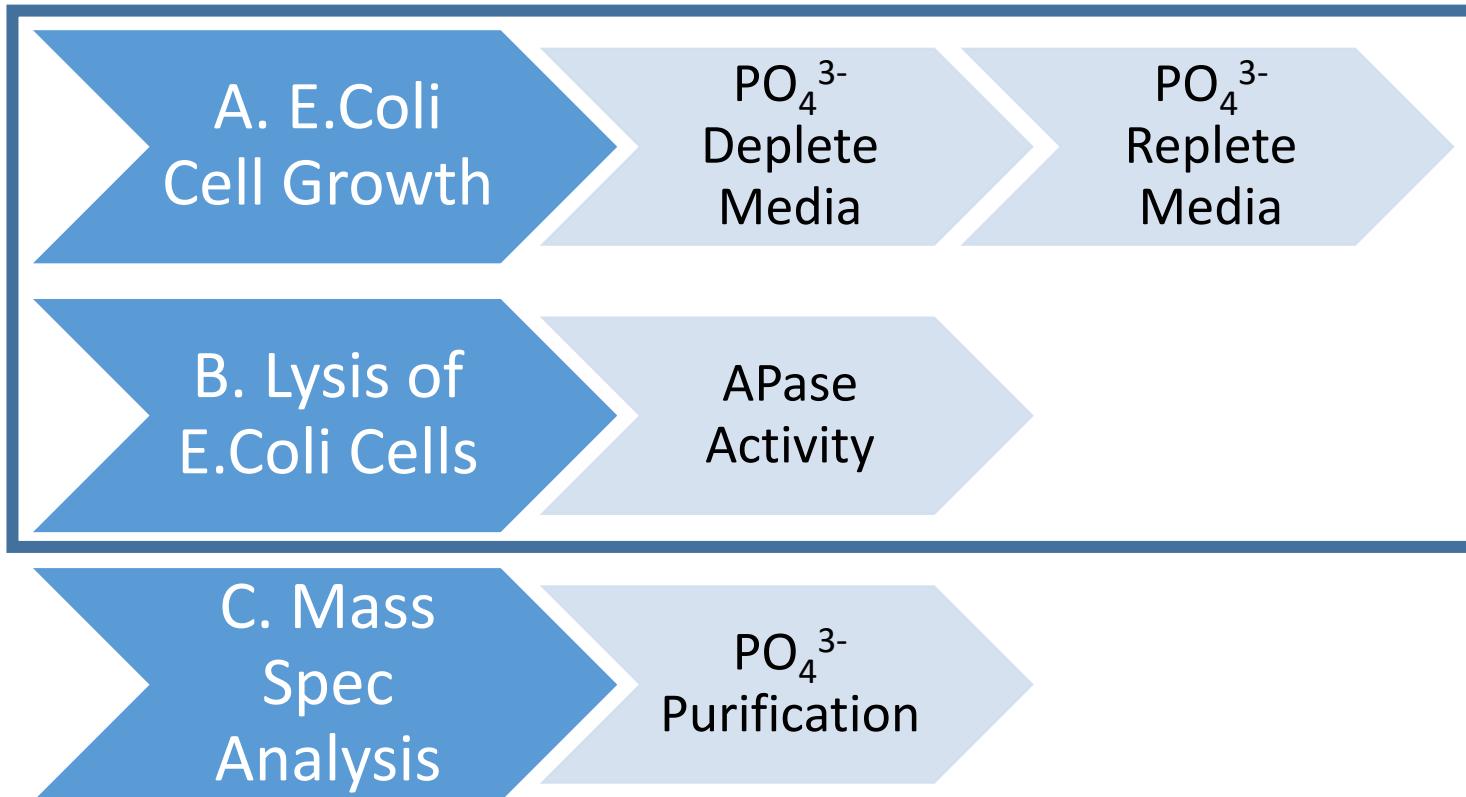


Background



II. Experimental Overview

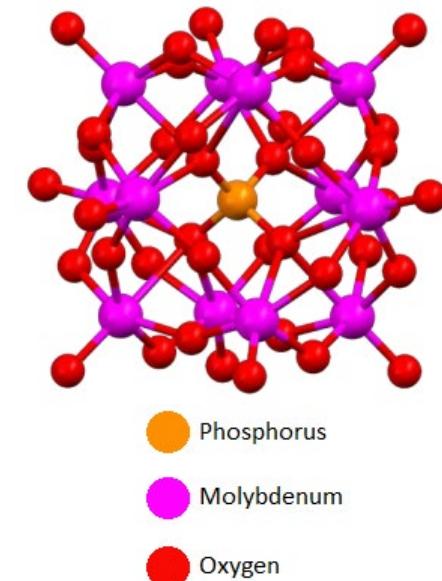
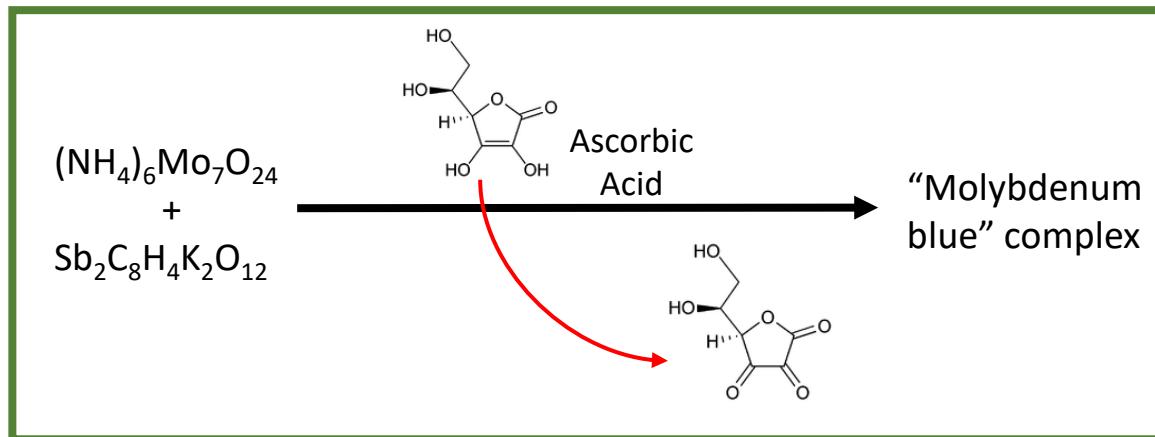
Experimental Overview



III. Analytical Methods

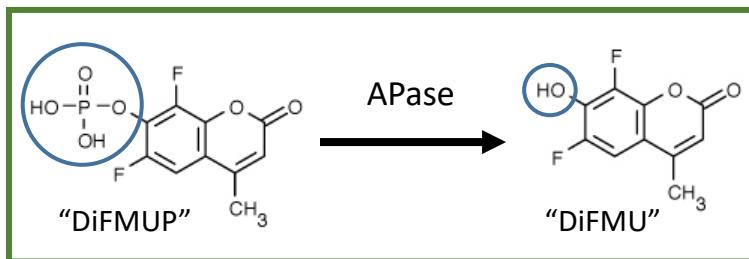
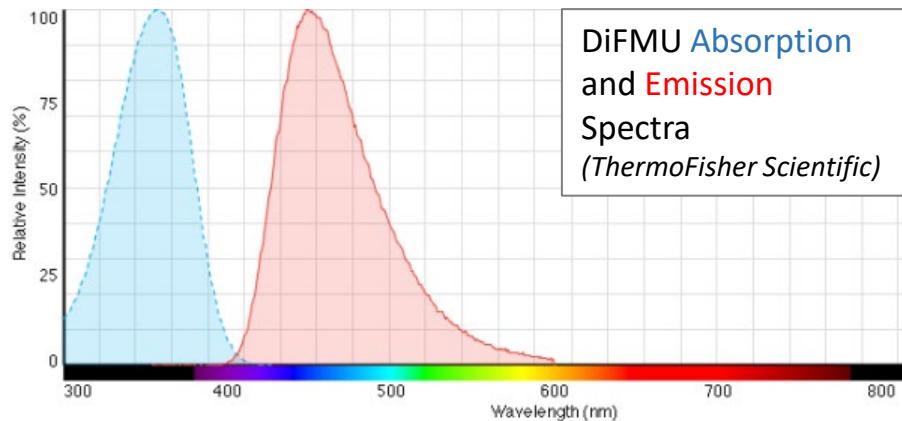
Spectroscopic Quantification of Pi

- 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

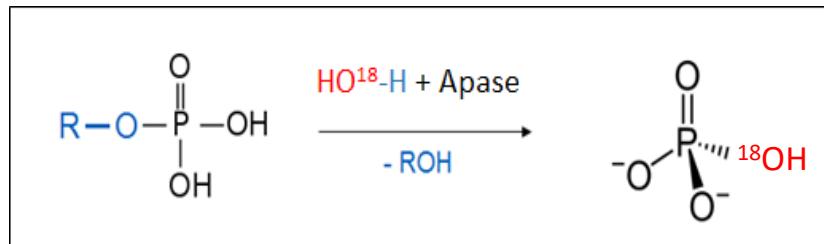
- Cleavage of H_2PO_4^- from the DOP compound "DiFMUP" produces the fluorescent "DifMU" product



Stable Isotope Labeling & Mass Spec.

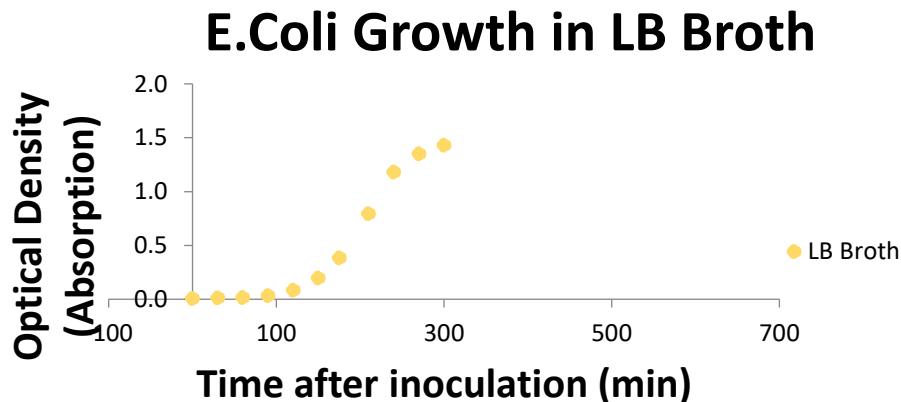
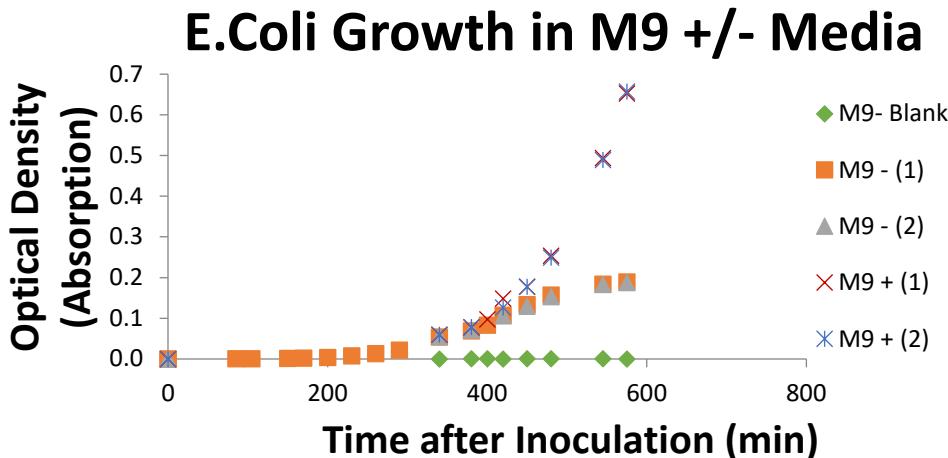
1 H Hydrogen					7 N Nitrogen					2 He Helium
3 Li Lithium	4 Be Beryllium				6					
11 Na Sodium	12 Mg Magnesium				5					
19 K Potassium	20 Ca Calcium	21 Sc Scandium	22 Ti Titanium	23 V Vanadium	24 Cr Chromium	25 Mn Manganese	26 Fe Iron	27 Co Cobalt	28 Ni Nickel	5 B Boron
37 Rb Rubidium	38 Sr Strontium	39 Y Yttrium	40 Zr Zirconium	41 Nb Niobium	42 Mo Molybdenum	43 Tc Technetium	44 Ru Ruthenium	45 Rh Rhodium	46 Pd Palladium	6 C Carbon
55 Cs Cesium	56 Ba Barium	57 La Lanthanum	72 Hf Hafnium	73 Ta Tantalum	74 W Tungsten	75 Re Rhenium	76 Os Osmium	77 Ir Iridium	78 Pt Platinum	7 N Nitrogen
87 Fr Francium	88 Ra Radium	89 ** Ac Actinium	104 Rf Rutherfordium	105 Db Dubnium	106 Sg Seaborgium	107 Bh Bohrium	108 Hs Hassium	109 Mt Meitnerium	110 Ds Darmstadtium	13 Al Aluminum
+ 58 Ce Cerium	59 Pr Praseodymium	60 Nd Neodymium	61 Pm Promethium	62 Sm Samarium	63 Eu Europium	64 Gd Gadolinium	65 Tb Terbium	66 Dy Dysprosium	67 Ho Holmium	68 Er Erbium
** 90 Th Thorium	91 Pa Protactinium	92 U Uranium	93 Np Neptunium	94 Pu Plutonium	95 Am Americium	96 Cm Curium	97 Bk Berkelium	98 Cf Californium	99 Es Einsteinium	100 Fm Fermium
										15 P Phosphorus
										16 S Sulfur
										17 Cl Chlorine
										18 Ar Argon
										31 Ga Gallium
										32 Ge Germanium
										33 As Arsenic
										34 Se Selenium
										35 Br Bromine
										36 Kr Krypton
										51 Te Tellurium
										52 I Iodine
										53 Xe Xenon
										81 Tl Thallium
										82 Bi Bismuth
										83 Po Polonium
										84 At Astatine
										85 Rn Radon
										115 Uup Ununpentium
										116 Uus Ununhexium
										117 Uuo Ununoctium
										101 Md Mendelevium
										102 No Neptunium
										103 Lr Lawrencium
										70 Yb Ytterbium
										71 Lu Lutetium

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



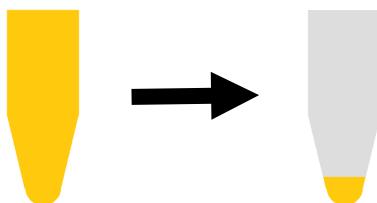
IV. Results

A. Cell Growth

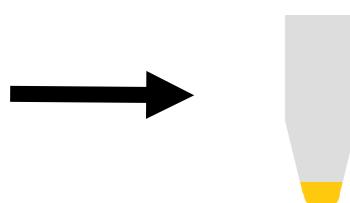
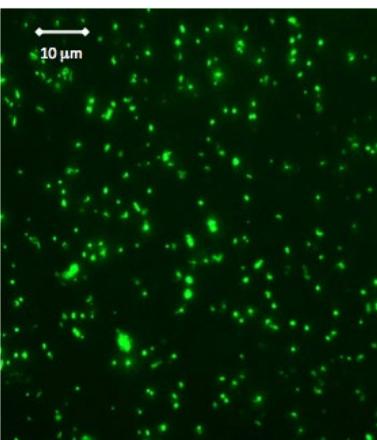


- Growth Media
 - M9+ (Pi Replete)
 - 700mM Pi
 - M9- (Pi Deplete)
 - 5.8mM GYP
 - 5.8uM Pi
- M9- selects for APase expression
 - DOP dominates as source for P
- *E.Coli* K-12 MG1665

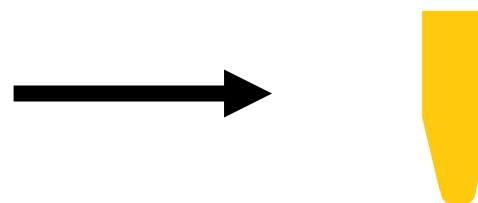
B. Cell Lysis



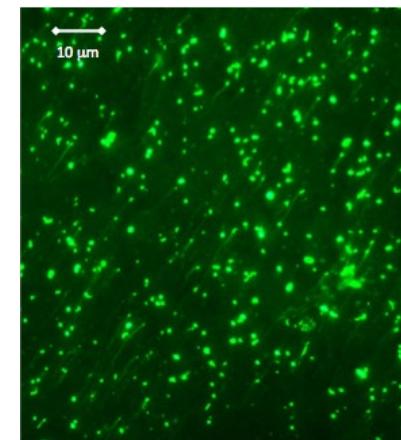
Cells Centrifuged



Supernatant
Removed,
Cells washed



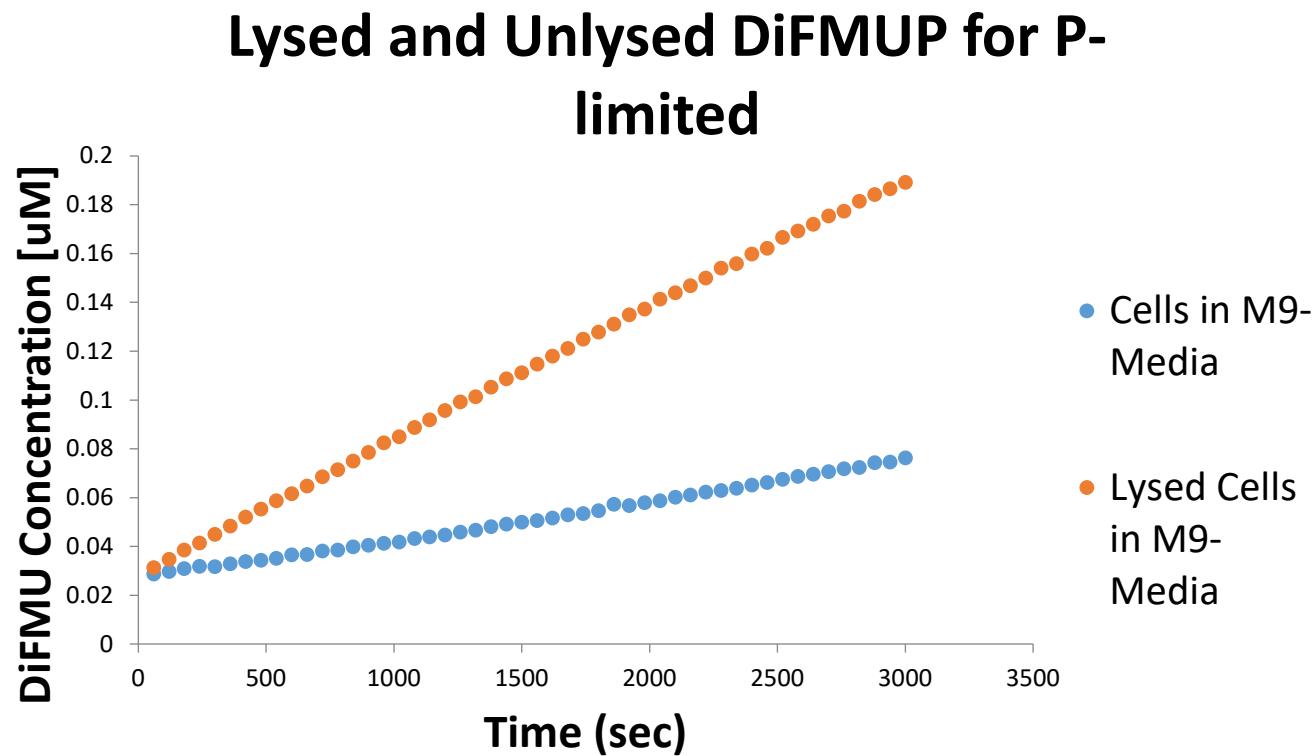
Cells Re-suspended
in lysozyme



Courtesy of Aric
Mine

Results

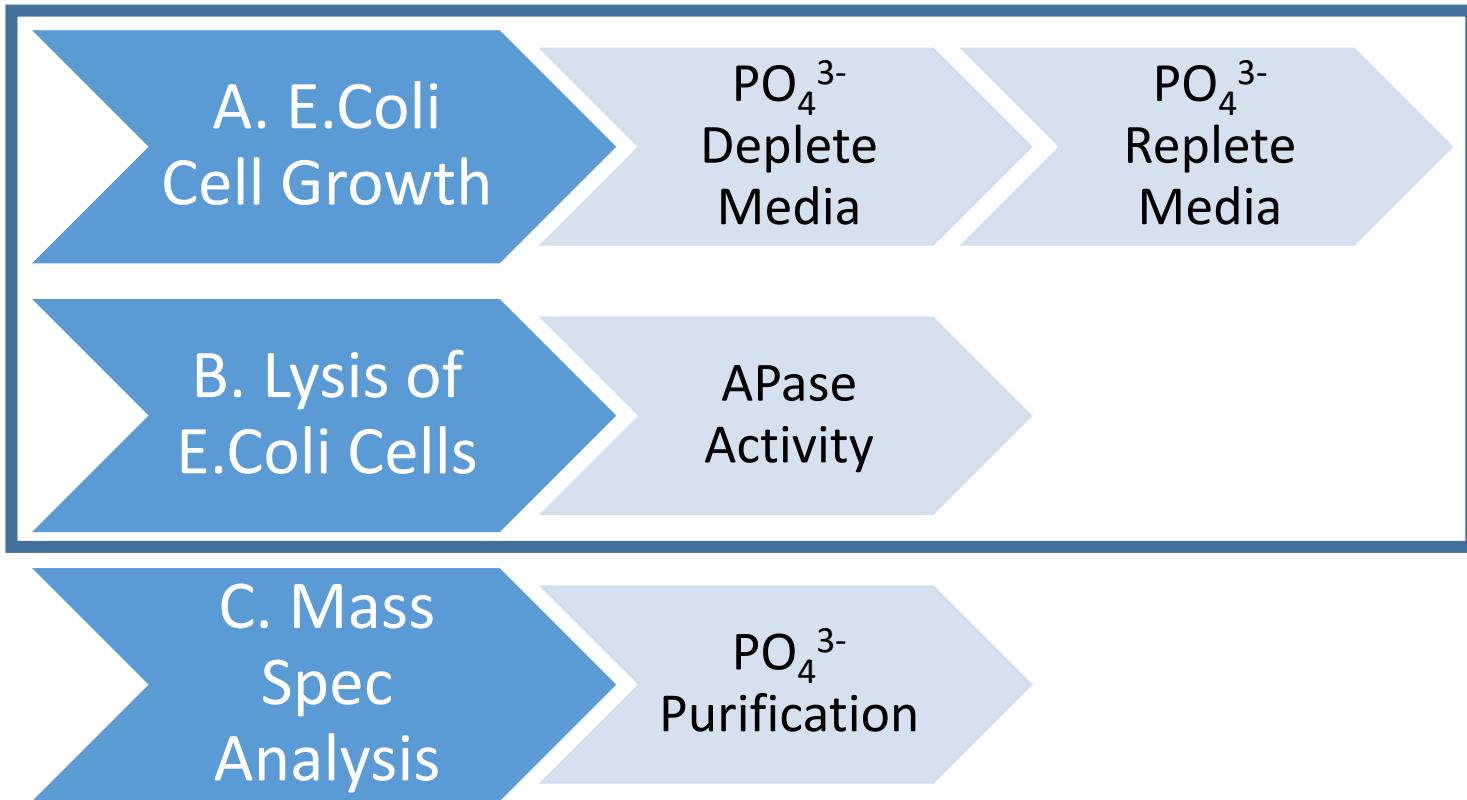
C. Cell Lysis and APase Activity



Results

V. Future Directions

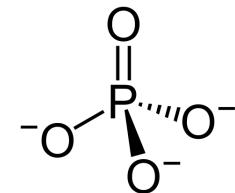
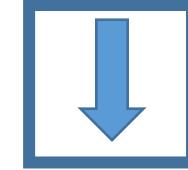
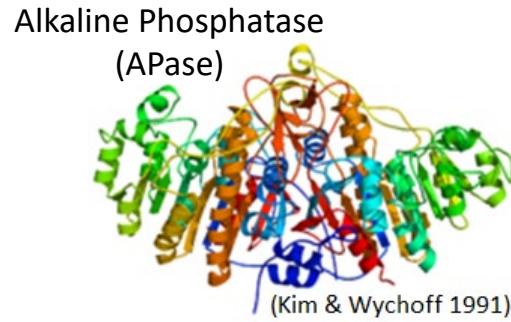
V. Future Direction



Future Direction

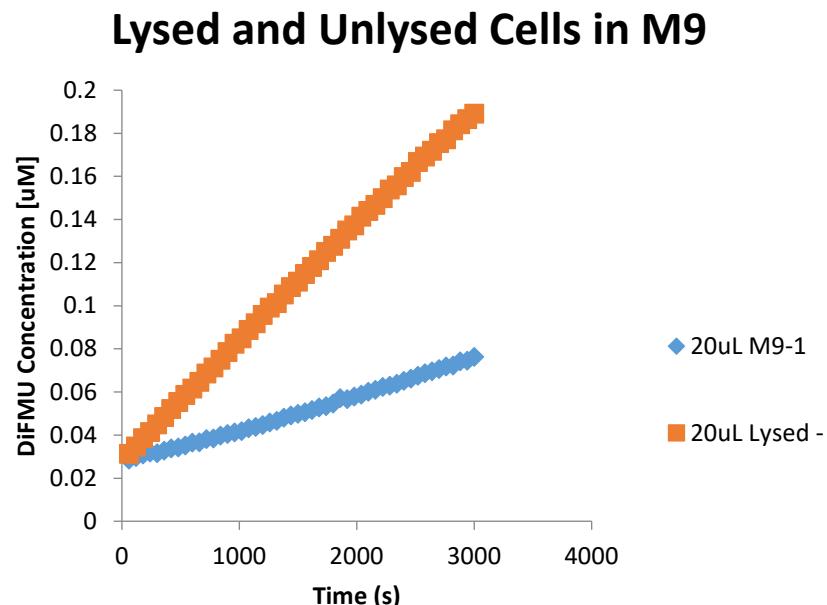
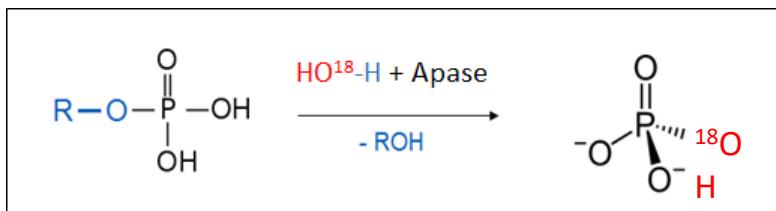
Future Direction: Pi isolation from M9 media

- 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



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



Acknowledgements

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