

Studying the Electrochemical Activity of Methanol Dehydrogenase in Lanthanide-Modified *Methylobacterium Exorquens*

USING BACTERIA TO CONDUCT ELECTRICITY - BIOELECTROCATALYSIS

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INTRODUCTION

Methylobacterium exorquens are a species of bacteria known to oxidize methanol via the enzyme methanol dehydrogenase (MDH), which typically has a Ca^{+2} metal cofactor (MxaFI) but can be induced to use rare earth metals like lanthanum (La^{+2}) as a metal cofactor (XoxF1). Nonelectrochemical studies show that *M. exorquens* with La^{+2} MDH have higher activity than *M. exorquens* with Ca^{+2} MDH. Current research seeks to prove through both electrochemical and kinetic techniques that lanthanum is more oxidizing than the calcium.

PROCEDURE

1. *M. exorquens* are cultured by incubation in LB media enriched with the desired cofactor until the colonies reach log phase
2. Cells are harvested by high-speed centrifugation and flash-frozen for storage at -80°C . Optical densities (OD) are taken throughout each generation
3. Cell samples were thawed for use in DCPIP-PMS coupled assays in 100 mM Tris-HCl buffer (pH 9.00), 2mM DCPIP and 100mM PES were added to bacteria and the substrate methanol, the concentration was varied between 1mM and 10mM.

FILMS/MEDIATORS

Films are needed to immobilize bacteria on an electrode, and mediators are necessary for electron transfer in the reduction-oxidation process. Listed below are some of the films and mediators that have been experimented with:

FILMS:

1. Biofilm (no polymer)
2. Chitosan
3. Polyvinyl Alcohol
4. Polyethylene Glycol

MEDIATORS:

1. Methyl Viologen (in solution and electropolymerized)
2. Methylene Green
3. Cytochrome C



RESULTS

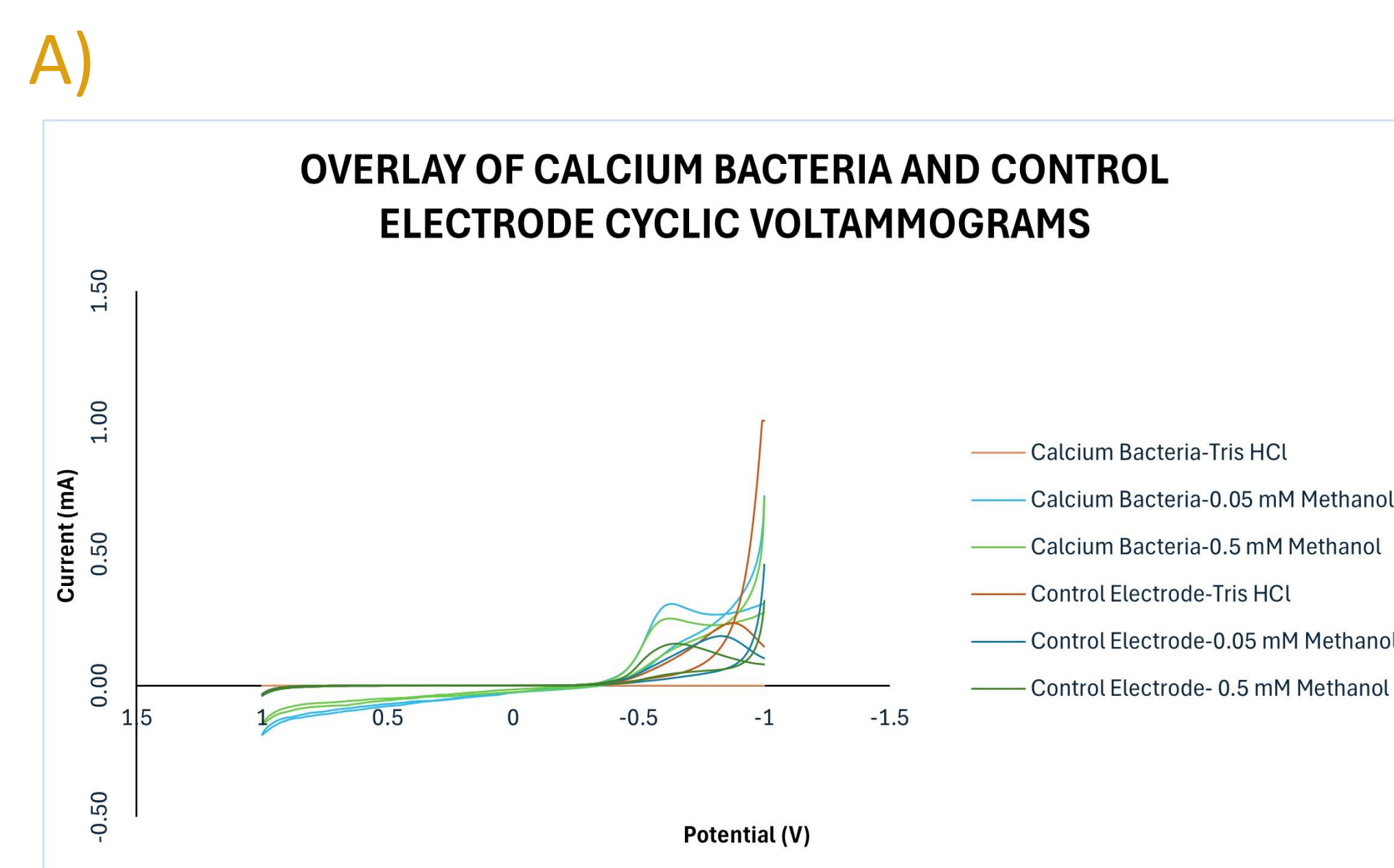


Figure A: Cyclic Voltammogram overlay of Calcium Bacteria on a Carbon Paper Electrode and a Control Carbon Paper Electrode in 100 mM Tris-HCl buffer (pH 9.00). Different colors represent the different electrodes and increasing concentrations of methanol as indicated.

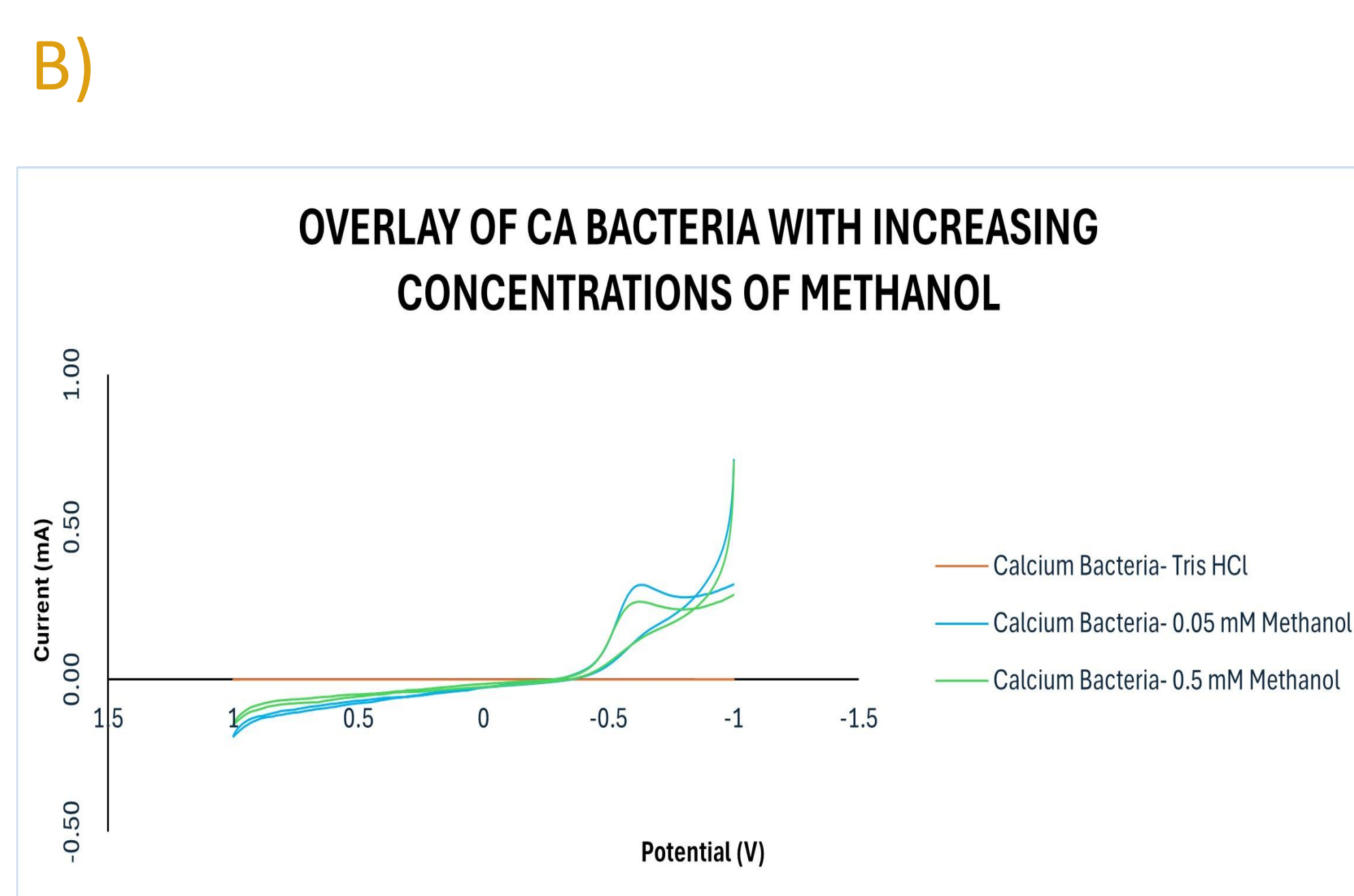


Figure B: Cyclic Voltammogram overlay of Calcium Bacteria on a Carbon Paper Electrode in 100 mM Tris-HCl buffer (pH 9.00). Different colors represent increasing concentrations of methanol as indicated.

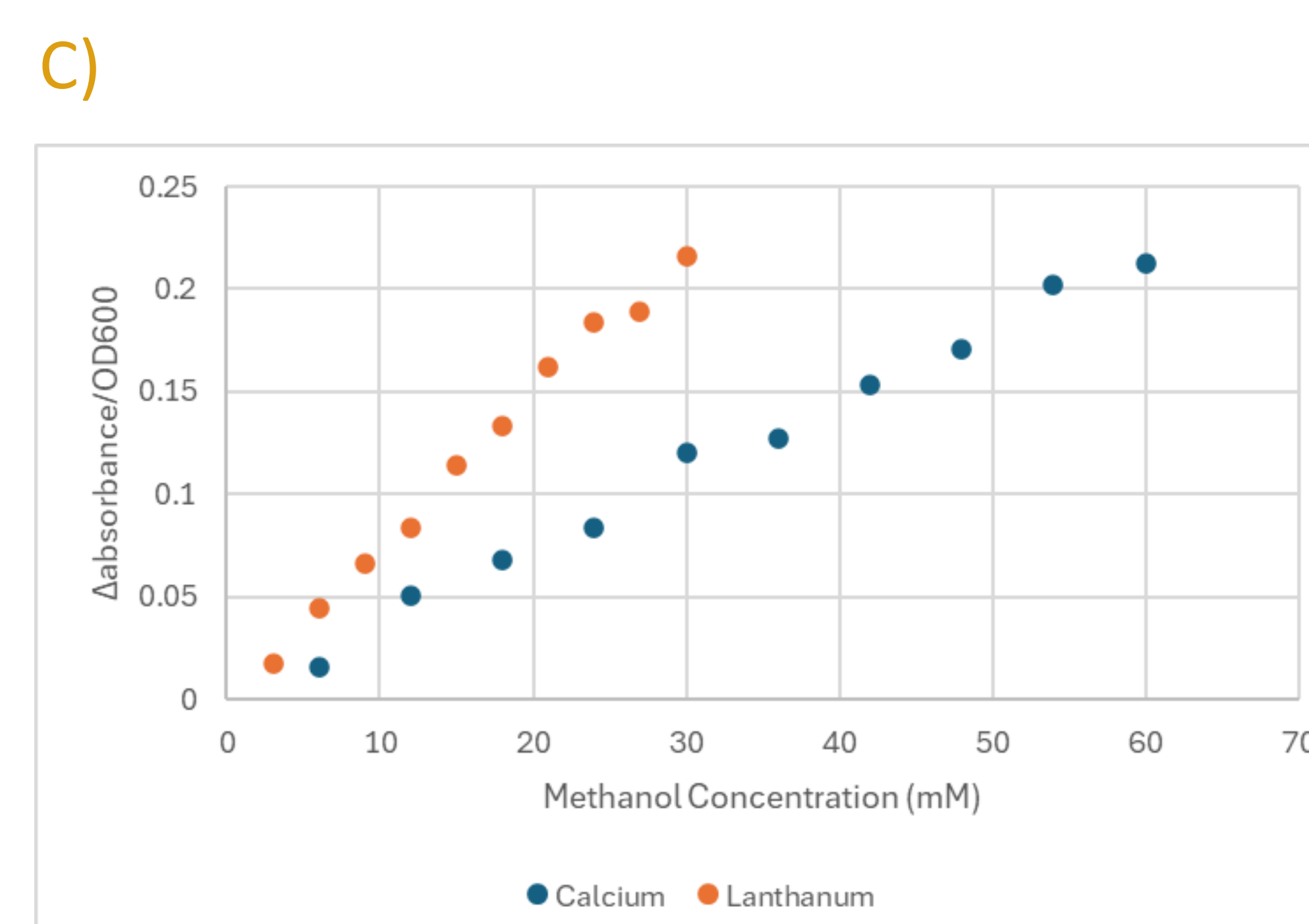
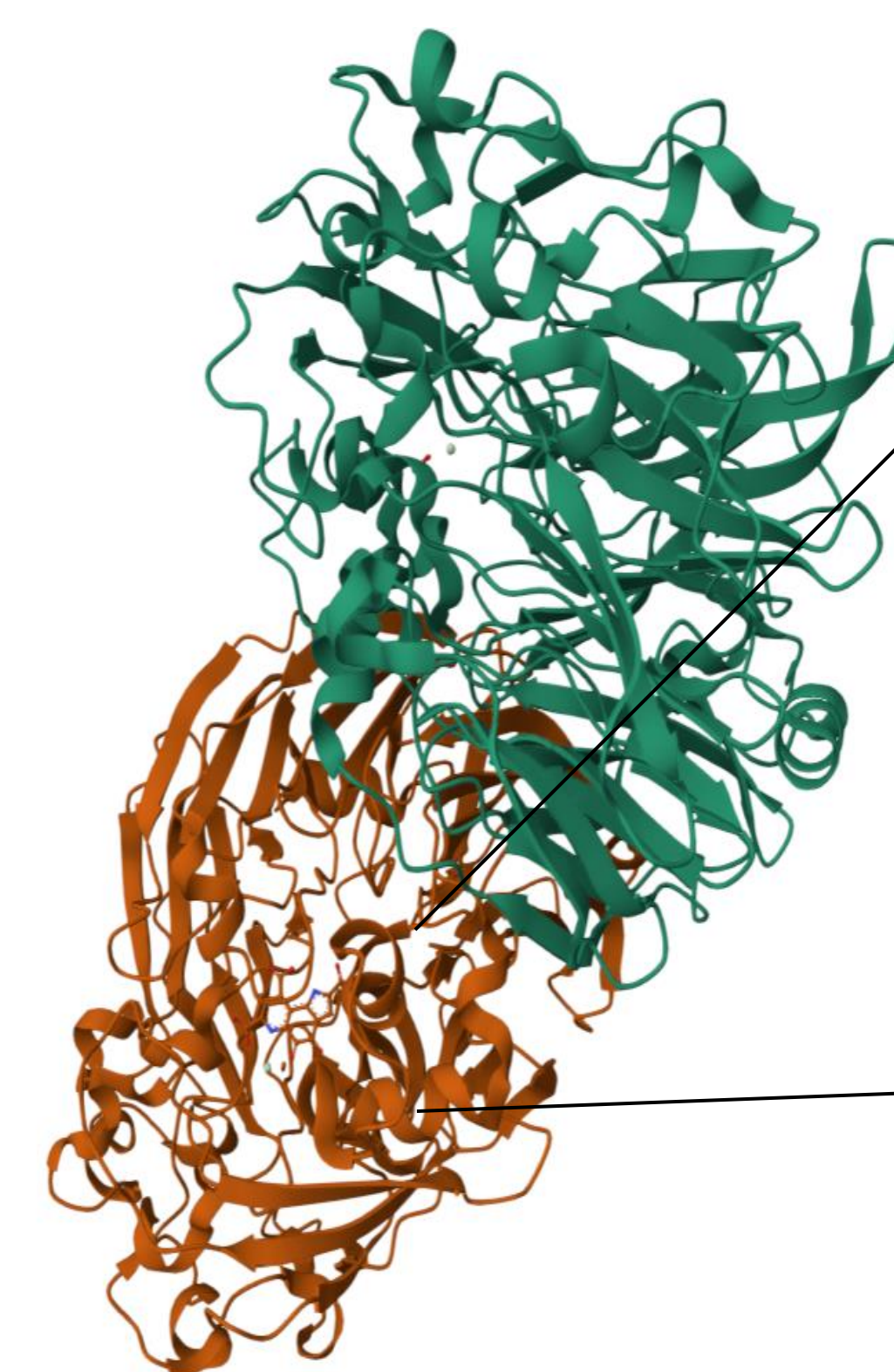
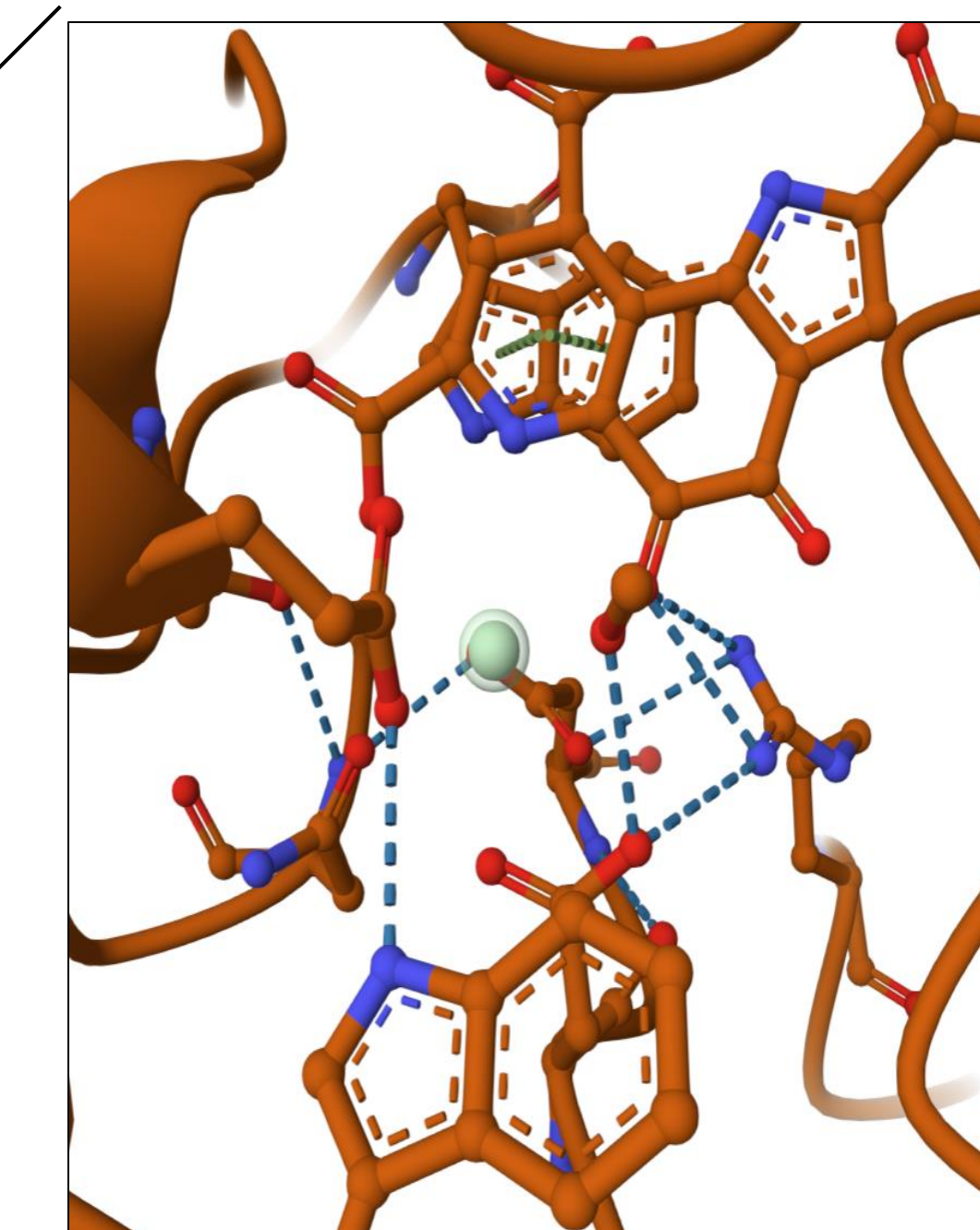


Fig C. DCPIP-PES coupled assay of 21st generation calcium bacteria (OD600 = 0.456) and lanthanum bacteria (OD600 = 0.561) with increasing methanol concentration.

D)

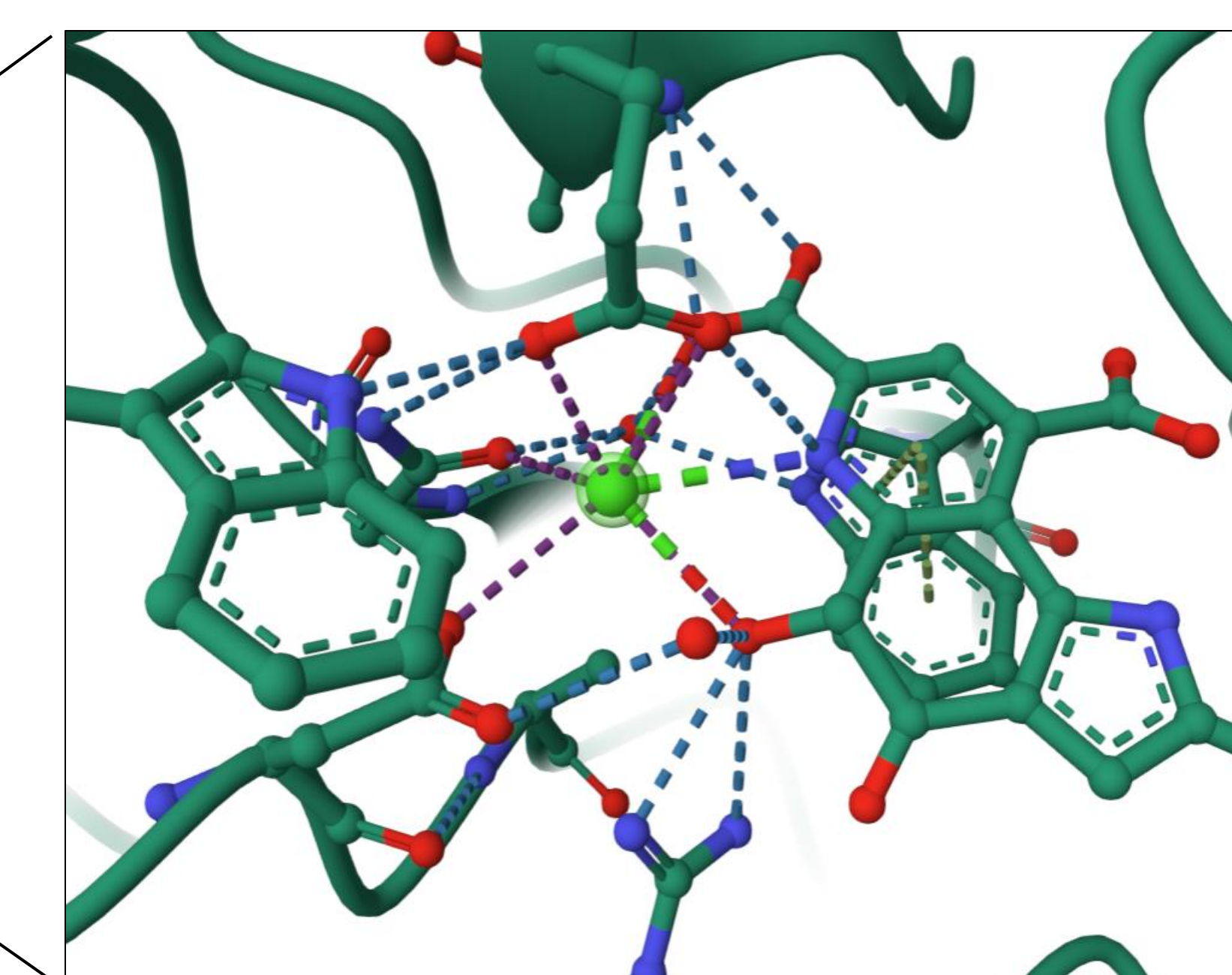
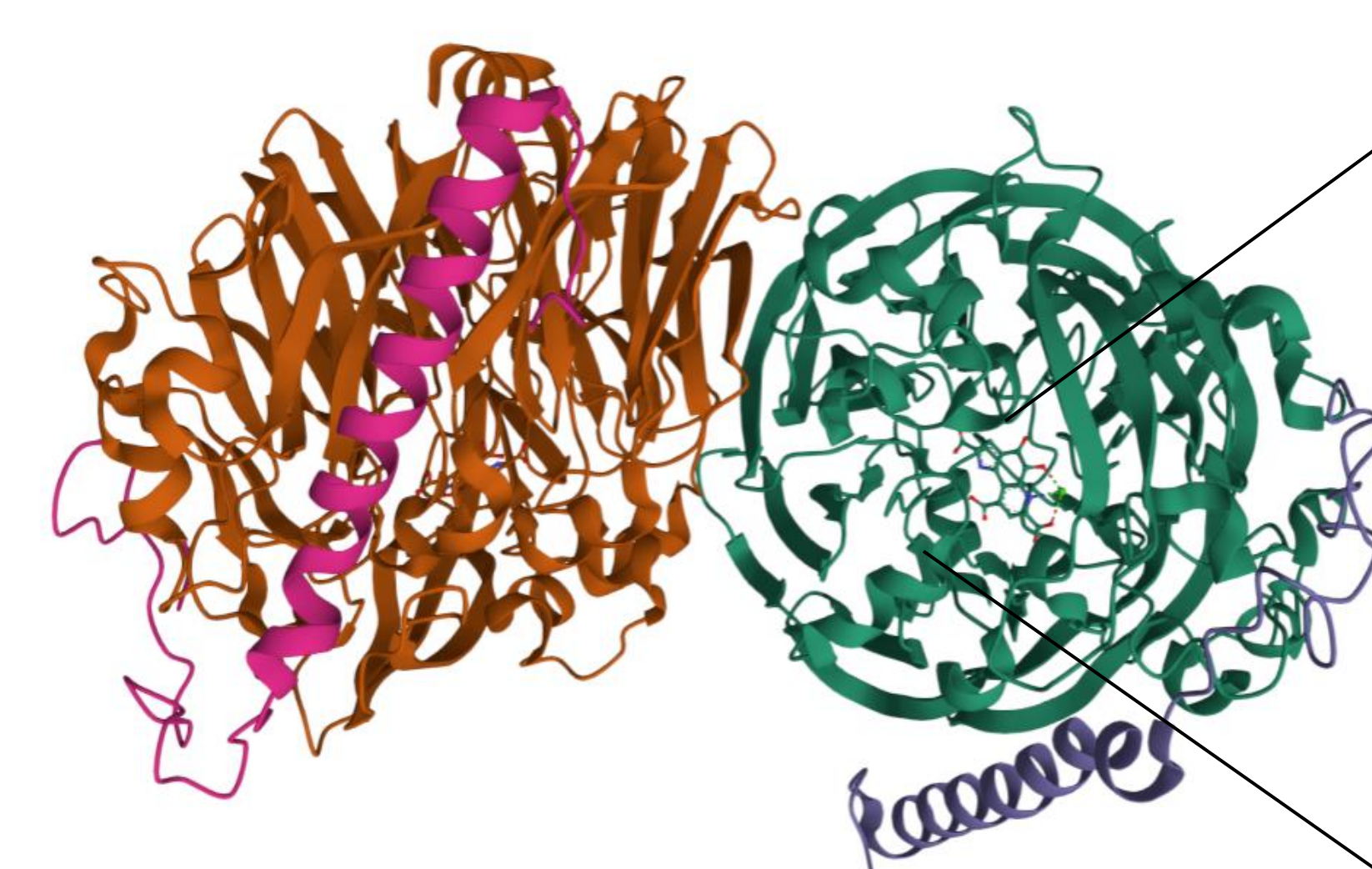


F)



Figures D and F: Structure of La-utilizing MDH dimer (XoxF) and active site. Primary components are the lanthanide ion (pale green) and pyrroloquinoline quinone cofactor (orange). (Li, J., Gan, J. H., Mathews, F. S., & Xia, Z. X. (2011). The enzymatic reaction-induced configuration change of the prosthetic group PQQ of methanol dehydrogenase. Biochemical and biophysical research communications, 406(4), 621–626. <https://doi.org/10.1016/j.bbrc.2011.02.107>)

E)



Figures E and G: Structure of Ca-utilizing MDH dimer (MxaFI) and active site. Primary components are calcium ion (neon green) and pyrroloquinoline quinone cofactor (orange). (Li, J., Gan, J. H., Mathews, F. S., & Xia, Z. X. (2011). The enzymatic reaction-induced configuration change of the prosthetic group PQQ of methanol dehydrogenase. Biochemical and biophysical research communications, 406(4), 621–626. <https://doi.org/10.1016/j.bbrc.2011.02.107>)

DISCUSSION AND FUTURE WORK

Work to-date has not yielded significant results in terms of mediation of methanol oxidation of MxaFI (Ca cofactor) or XoxF1 (La cofactor).

Future work on this project will be directed towards immobilizing the bacteria on the electrode and finding effective polymerized mediators, as well as optimizing the DCPIP-PMS coupled assays.

Further purification of bacteria by FPLC will be done to yield more conclusive results regarding the target enzyme. This will help understand the role of MDH in the electrogenesis of *M. exorquens*.

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