Structural study of soluble ammonia monooxygenase

Trevor Obrinsky[1], Alex McGurk[1], Hasan DeMirci[1,2]

[1]PULSE Institute.

[2]Linac Coherent Light Source, SLAC National Accelerator Laboratory, 2575 Sand Hill Road, Menlo Park, CA 94025, USA.

[+]Contact: hasan_demirci@stanford.edu

Introduction

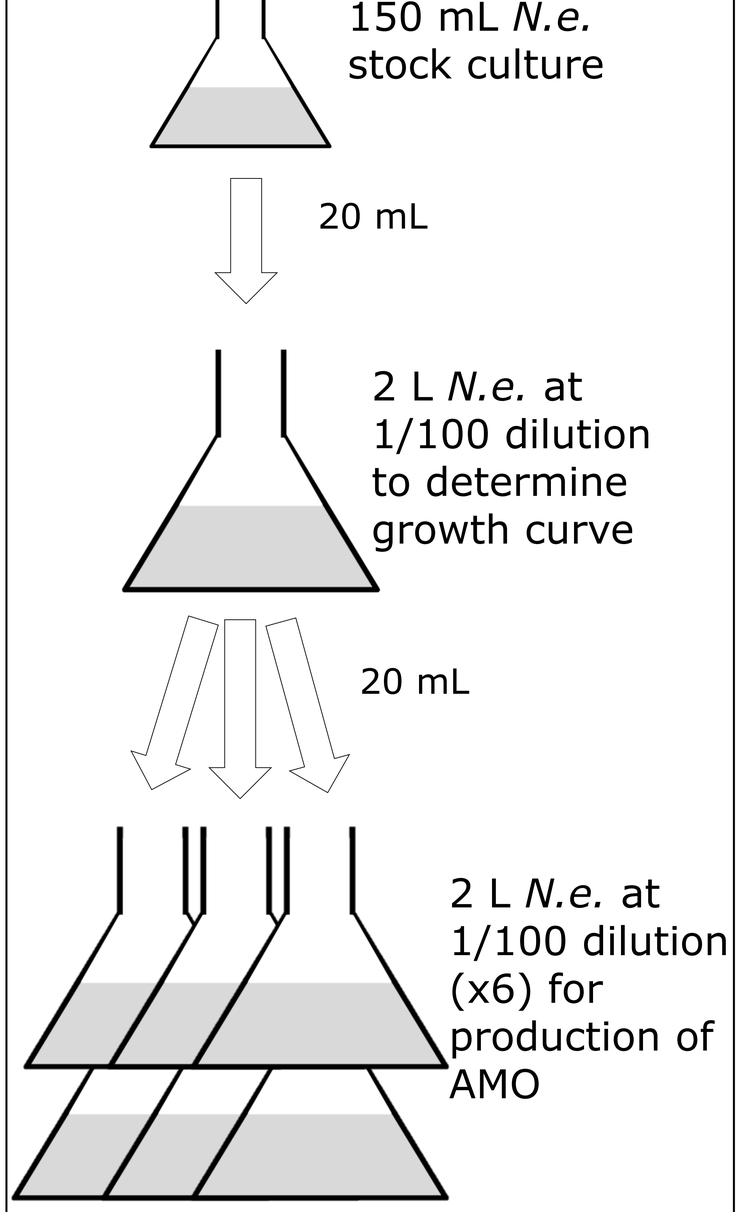
What makes *Nitrosomonas europaea* (*N.e.*) so unique is its ability to generate all it's required energies from the two step conversion of ammonia into nitrite. Ammonia monooxygenase (AMO) is the first of two enzymes in *N.europaea* responsible for the oxidation of ammonia into nitrite. AMO catalyzes the conversion of ammonia into the hydroxylamine intermediate.

$$NH_3 + O_2 + 2H^+ + 2e^- \xrightarrow{AMO} NH_2OH + H_2O$$

Ammonia is useless to most organisms but this conversion into nitrite (part of the nitrogen cycle) makes it accessible to other organisms that need it for macromolecular synthesis. We are interested in *Nitrosomonas europaea* and the structure of ammonia monooxygenase due to its crucial role in the nitrogen cycle.

Keywords: *Nitrosomonas europaea*, ammonia monooxygenase, nitrogen cycle

Research



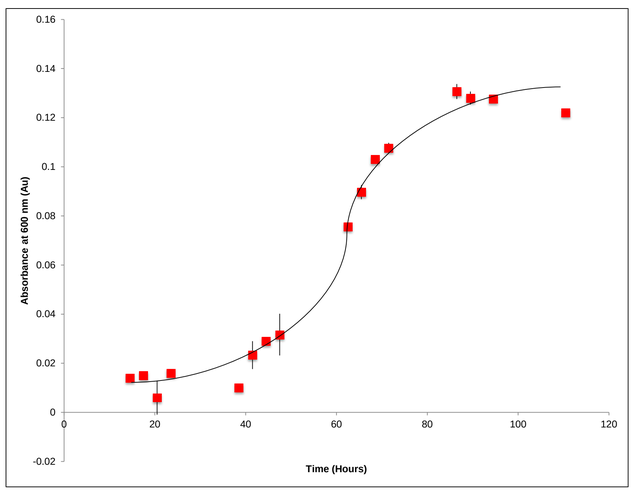


Fig. 1: Growth curve of *Nitrosomonas europaea* used to determine ideal time to harvest *N. europaea* cells for maximum AMO concentration

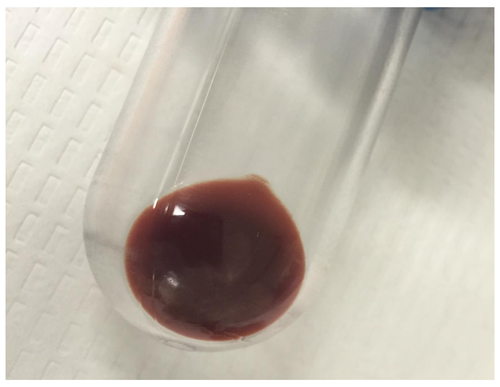
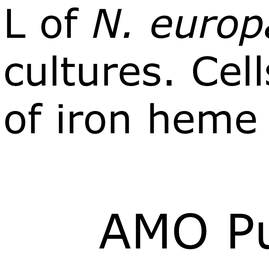


Fig. 2: Total cell paste from 12 L of *N. europaea* growth cultures. Cells are red because of iron heme groups.

AMO Purification

Lyse cells with Emulsiflex

Ultracentrifuge



FPLC with multiple columns to isolate AMO

SDS-PAGE to confirm purification

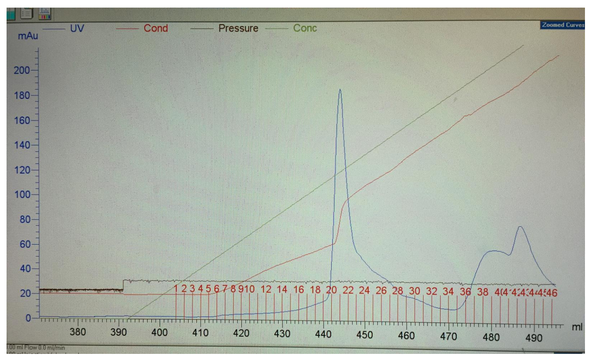


Fig. 3: Graph of absorbance, salt concentration, and conductance during the elution of protein in a strong anion exchange column. Absorbance peaks represent potential subunits of AMO. Bound protein was eluted across a 100 mL salt gradient from 0-1 M.

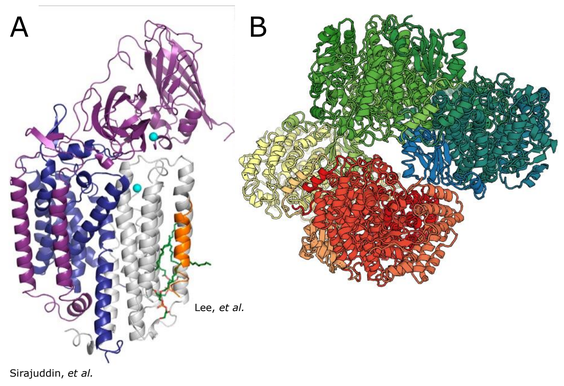


Fig. 4: A) Crystal structure of one protomer of membrane bound methane monooxygenase (pMMO) made up of pmoB, pmoA, and pmoC. B) Crystal structure of soluble MMO (sMMO). MMO and AMO are homologous enzymes.

Conclusions

We determined the growth curve of *N. europaea.* Literature showed that harvesting cells in late exponential phase maximized AMO in the cell. Future directions for this project include continuing the purification process for the remaining *N. europaea* cell pellets, finding suitable crystallization conditions for pure AMO, and using the crystals at a beam time to determine the crystal structure of AMO.

Acknowledgments

Use of the Linac Coherent Light Source (LCLS), SLAC National Accelerator Laboratory, is supported by the U.S. Department of Energy, Office of Science, Office of Basic Energy Sciences under Contract No. DE-AC02-76SF00515.

Date: 08/11/2016