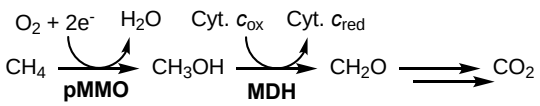
SPECIFIC AIMS

Anthropogenic climate change, driven in large part by methane emissions, damages human health by contributing to generalized climate instability, ozone accumulation, and future pandemics (1, 4-6). Atmospheric methane is regulated by methanotrophs, bacteria that consume methane using methane monooxygenases (MMOs) (7-9). One promising route toward mitigating the burdens of methane emissions on human health exists in the particulate methane monooxygenase (pMMO), a copper-dependent transmembrane enzyme that converts methane to methanol (**Eq. 1**), a desirable liquid biofuel (10, 11). Previous studies of pMMO have been impeded by a loss of activity and stability upon removal and detergent solubilization from the membrane (12). Detergent micelles do not recapitulate the physicochemical properties of the native membrane, causing distortions to hydrophobic transmembrane regions of the enzyme, which may not be entirely reversible (13). Activity may be partially recovered upon reconstitution of pMMO into bicelles or nanodiscs, and this reversible loss and subsequent recovery of activity is not associated with the loss or recovery of copper, respectively (12-14). These findings suggest that the membrane, or membrane mimetic, environment itself contributes to the structural and functional stability of pMMO.

Equation 1: Oxidation of methane by pMMO and methanol by MDH.



The guiding hypothesis of this project is that the membrane plays a critical role in supporting the structure and function of pMMO, likely through both specific protein-lipid interactions and general membrane properties. Put simply, the Specific Aims of this project are as follows: 1) assemble native membrane mimetics, 2) characterize their contents, and 3) visualize pMMO in their structures. The purpose of understanding the role of the membrane in pMMO structure and function is twofold. First, constructing better membrane mimetic systems for pMMO will make the enzyme more amenable to biophysical and biochemical study, providing an avenue for understanding its catalytic mechanism and the important factors for optimal activity. Second, understanding how the membrane environment stabilizes pMMO structure and function may reveal generalizable properties about membrane proteins and membrane mimetics, addressing a significant gap in knowledge.

Aim 1: Optimize pMMO activity in detergent-free native nanodisc systems.

Hypothesis: The membrane environment surrounding pMMO has a direct effect on its function. To test this hypothesis, pMMO activity will be compared in synthetic lipid nanodiscs, native lipid nanodiscs, and detergent-free native nanodiscs. Preliminary data show that pMMO can be reconstituted in native lipid nanodiscs that exhibit activity rivaling or surpassing that of pMMO in synthetic lipid nanodiscs. Polymer-based nanodisc systems such as styrene-maleic acids (SMAs) or diisobutylene-maleic acids (DIBMAs) are able to extract membrane proteins from the native membrane in a detergent-free manner, sparing the protein from detergent-driven distortions and preserving the native microenvironment. Detergent-free native nanodisc systems will be optimized for use with pMMO in order to delineate the effects of detergent solubilization on pMMO activity. More native-like platforms for pMMO will also enable protein-lipid interactions that may be important for its function.

Aim 2: Characterize of the membrane environment and its interaction with pMMO.

Hypothesis: The specific lipid composition of the native membrane is important for the function of pMMO. To test this hypothesis, the lipid environments of membranes and mimetics will be determined. Expected outcomes are: 1) general composition of the lipid environment, 2) specific identification of abundant lipids, and 3) specific protein-lipid interactions with pMMO. In order to test both specific and generalized lipid effects on pMMO, activity will be compared in nanodiscs containing homogenous and mixed lipid environments that reflect the composition of the native membranes. This information will reveal which lipid compositions are most beneficial for its function. Understanding how surrounding lipids interact with pMMO will inform mimetic design and the modeling of protein-lipid interactions in high-resolution crystal and cryoEM structures, particularly in crucial transmembrane regions.

Aim 3: Characterize of the structural effects of membrane mimetic environments on pMMO.

Hypothesis: More native-like membrane mimetics will stabilize the transmembrane regions of pMMO while supporting putative protein-lipid and protein-protein interactions. To test this hypothesis, structures of pMMO in native membrane mimetics will be determined to shed light on which membrane-related factors are most relevant for its structural stability. Native membrane mimetics will reconstitute specific protein-lipid interactions that stabilize these transmembrane regions and the metal centers contained within them. pMMO has also been hypothesized to form a methane-oxidizing supercomplex with methanol dehydrogenase (MDH), the next enzyme in the methane metabolism pathway (**Eq. 1**). MDH associates with the membranes, suggesting that a membrane-like environment is required for stabilizing its proposed interaction with pMMO. Capturing this interaction may reveal methanol or electron transfer pathways from the currently unidentified pMMO active site. More native-like structures of pMMO will provide a more complete picture of the enzyme, which will be crucial for elucidating its mechanism of methane oxidation.

This proposal centers on the application of innovative membrane mimetic and structural technologies for studying pMMO, addressing decades-old questions about this important enzyme and membrane proteins in general. The scientific research and training program described will allow me to pursue interdisciplinary research at the cutting-edge, furthering my potential to make positive impacts as a scientist.

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