Goldschmidt 2022 Abstract
https://doi.org/10.46427/gold2022.12546

Bacterial Clathrate-Binding Proteins in the Deep Subsurface Biosphere: Implications for Gas Clathrate Stability and Habitability

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Gas clathrates, high-pressure habitats that naturally occur on continental shelves and in permafrost, host microbial communities unique from other subsurface ecosystems. Gas clathrates are a natural gas resource and can also serve as Earth analogs for habitability elsewhere in our solar system, such as Mars and Titan. The habitability and biosignatures of microbial activity in methane clathrates remain virtually unstudied. Ice-binding proteins enable diverse life to survive at low temperatures and colonize ice. In addition to ice, ice-binding proteins also bind and induce structural changes to gas clathrates. To date, the most effective ice-binding proteins tested for clathrate inhibition are type I winter flounder antifreeze proteins. Bacteria living in gas clathrates may employ a strategy like that of winter flounder that express antifreeze proteins to inhibit the growth of ice crystals. We identified bacterial **C**lathrate-**B**inding **P**roteins sequenced from gas clathrate-bearing sediments based on their similarity to ice-binding proteins. By synthesizing clathrate in the presence of recombinantly expressed CBPs, we found that these CBPs bind and alter the structure of tetrahydrofuran clathrate, a structure II clathrate. We then tested the effect of those CBPs on methane clathrate, a structure I clathrate. A methane clathrate shell was formed on a treatment droplet containing 13 µM protein at 5 MPa and -10ËšC. Significantly less methane clathrate formed in the presence of CBPs and the commercial gas clathrate inhibitor, PVP, relative to negative controls (cytochrome and PBS), as measured by gas consumption. Additionally, the winter flounder antifreeze proteins did not significantly alter methane clathrate formation compared to negative controls at the high driving force used in this study. Clathrate morphology in treatments with less clathrate formed dome-shaped shells, whereas treatments with more clathrate formed cratered shells. Overall, we found that the CBPs alter methane clathrate morphology and inhibit methane clathrate formation similarly to the commercial inhibitor, polyvinylpyrrolidone. The bacterial proteins discovered in this study may aid in maintaining habitable environments for bacteria in these high-pressure clathrate systems.

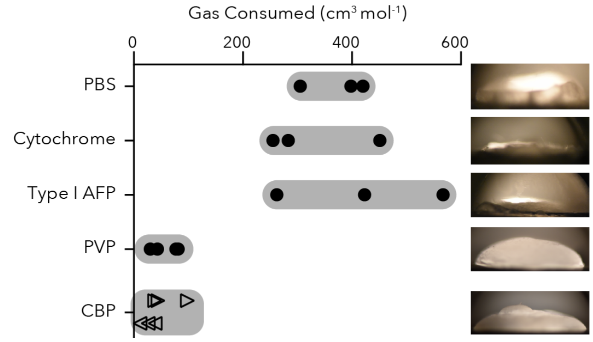

Figure 1. CBPs inhibit methane gas consumption as well as the commercial gas clathrate inhibitor, polyvinylpyrrolidone (PVP), both of which result in dome-shaped methane clathrate shells.