

## Genetically-Engineered Mutant Antifreeze Proteins Provide Insight Into Hydrate Inhibition

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Perhaps curiously, certain antifreeze proteins (AFPs) act as kinetic inhibitors for gas hydrate growth, and it is speculated that this may be mediated by an adsorption-inhibition mechanism. To test this hypothesis, AFPs 'tagged' with a green fluorescent peptide (GFP) have been used to examine the inhibition of natural gas hydrates, as well as the adsorption to polycrystals of a model clathrate, tetrahydrofuran (THF) hydrate. Steric mutations of the grass, *Lolium perenne* (*Lp*), AFP can interfere with ice adsorption and AFP activity if these are located on a particular 'flat' face, thereby positively identifying a single ice-binding face (IBF) on this protein. 'IBF mutations' on a recombinant *Lp*AFP-GFP disrupted ice adsorption as expected, but the mutant protein still adsorbed to polycrystalline hydrate with an affinity similar to the nonmutant *Lp*AFP-GFP. A *Lp*AFP-GFP with a mutation on the opposite, non-IBF side adsorbed to both polycrystalline ice and THF hydrate. These experiments coupled with our recent studies on AFP-THF hydrate single crystal morphology, have provided support and insight into the adsorption-inhibition of hydrate growth by these unique proteins.

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