Investigating the Inhibition Mechanism of THF Hydrates and Methods of Hydrate Growth

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Yeshiva University

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Tamar Nicole Soussana

Mentor: Dr. Ran Drori, Chemistry and Biochemistry

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1. Introduction

1.1 What are gas hydrates?

A gas hydrate, also known as a clathrate hydrate, is a crystal involving hydrogen bonded water molecules that form a cage around a single molecule, most commonly a gas such as methane or ethane (Fig. 1). These crystals can effectively reserve hydrocarbons at very high concentrations, accumulating an abundance of gas and energy.[1,2] The term hydrates here is in reference to hydrate crystals which are not to be confused with hydrates referring to compounds of stoichiometric ratio between water and various salts. However, there are ratios between the number of water molecules and the number of guest molecules in which these crystals likely form. Namely, 85 mol% of water molecules and 15 mol% of guest molecules.[2]

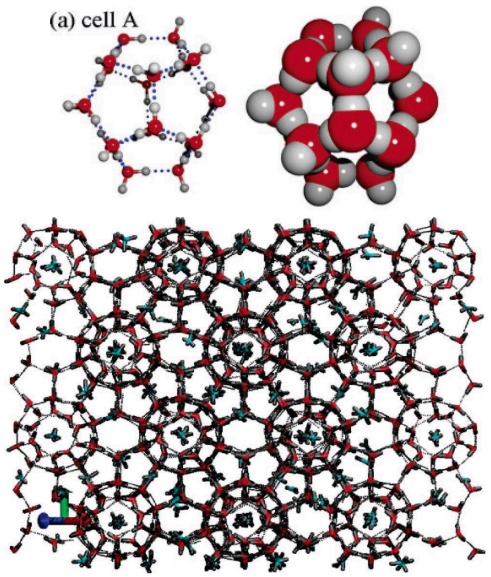


Figure 1. Molecular simulations of clathrate hydrates displaying their structure: a cage of water molecules encompassing a guest molecule. Taken from reference 5.

Clathrate hydrates form under conditions of low temperature and high pressure which are common in deep oceans, permafrost, or frozen grounds, and importantly, the petroleum industry.[1–3] The large presence of gas hydrates in these locations offers a potential energy source, larger than that of coal and oil.[4,5] However, the extraction of these hydrates as well as the accumulating concentration of hydrates in pipelines brings about a safety concern. Methane, the most abundant natural gas compound, commonly forms hydrates in oil pipelines. The solid, non-flowing crystals, which are denser than

typical hydrocarbons, cause blockages and lead to oil spills and explosions due to ignition of the concentrated, high energy, and highly volatile gases in the hydrates (Fig. 2).[3]

Figure 2. The growth of gas hydrates in an oil pipeline. (Image by Bill Schmoker, PolarTREC).

Properties of pipelines that cause them to be a more hospitable and probable environment for the nucleation of hydrates are the high velocities of gas streams, pressure pulsations due to compressors, and the existence of small hydrate crystals, or hydrate nuclei.[3] The latter influences the direction of liquid gas and surrounding water vapor to geometrically assort in order to successfully formulate hydrates. Agitation of the melt, or solution of gas and water molecules, from high velocity and pulsating pressure, can also spur the nucleation of hydrates.[3] This is because agitation increases the probability that the correct molecules will be positioned near each other to form hydrates.

The presence of these hydrates in drilling pipes poses an immediate danger to our environment and economy. Some of the largest oil spills, such as the explosion of the Piper Alpha oil platform in 1988, and the Deepwater Horizon disaster in the Gulf of Mexico in 2010, have been accredited to hydrocarbon release and hydrate build up, resulting in the death of innocent people, and economic ramifications such as the loss of natural resources and aquatic life. Additionally, oil and gas companies in the United States spend around $1 billion annually on hydrate prevention, as estimated in 2015.[4] With natural gas standing as the premium source of fuel worldwide, as well as the ability for these crystals to release methane into the atmosphere, it is also important to

understand their development and inhibition in order to minimize the effect on climate change.[2]

1.2 Clathrate hydrates inhibitors

To avoid damages, the pursuit of an effective method to inhibit the accumulation of clathrate hydrates in flowlines is underway. Current methods of hydrate growth inhibition involve thermodynamic hydrate inhibitors (THIs) such as alcohols and glycols, which interfere with hydrate formation by creating hydrogen bonds with water, and thereby compete with the formation of hydrates.[2] This effect of lowering the chemical potential of water causes a freezing point depression, lowering the temperature required to form the crystal.[5] Methanol injection, as an example, is used in industry to lower the formation of methane hydrates in pipelines. The effective concentration of these THIs is very high, and can take up to 50 volume %.[5] On top of THIs being very costly due to the high concentration required, they may also lead to environmental hazards themselves[2,4–6]. The release of highly concentrated alcohols into the surroundings in the event of a pipe burst can negatively affect the environment, as they are very toxic and flammable. Additionally, ethylene glycol, another thermodynamic inhibitor, possesses hazardous properties for aquatic organisms.[4] Another downside to the use of methanol injection is that, while the inhibition of hydrates usually occurs in the aqueous phase, some methanol can transition into the nonpolar phase due to its methyl group, causing them to be unhelpful as inhibitors.[2]

Rather than using thermodynamic inhibitors, low-dosage hydrate inhibitors (LDHIs), or kinetic hydrate inhibitors (KHIs), have been the focus of hydrate research. Since these inhibitors are much less expensive and take up only around 0.5 weight %,

they are a very attractive replacement for THIs.[5] Common KHIs include polyvinylpyrrolidone (PVP), and poly(N-vinylcaprolactam) (PVCap), which have displayed hydrate inhibition in past studies[5,7–9]. Another inhibitor is polyvinyl alcohol (PVA), however it possesses little to no inhibition capacity.[8] KHIs interact directly with the hydrate surface, unlike thermodynamic hydrate inhibitors. KHIs increase the amount of time it takes for hydrates to form as well as slow their growth rate, rather than change the thermodynamic conditions which influence crystal nucleation.[8] However, the mechanism by which these inhibitors function at hydrate surfaces is still under investigation, and will be expounded upon in this paper.

Antifreeze proteins (AFPs), which are known to inhibit ice crystals in organisms living below subzero temperatures, were also found to inhibit hydrates[4,6,8,10,11]. Antifreeze proteins have evolved in multiple species to prevent the rupturing of cells and vessels in those organisms living in subzero temperatures, due to the expansion of water as a frozen solid, and the sharp growth of ice crystals. The mechanism of these proteins on ice crystals can shed light onto the mechanism of other KHIs. Importantly, the use of AFPs in industry to prevent the accumulation of gas hydrates in oil pipelines could stand as a 'green inhibitor,' or environmentally safe way to solve this problem, as AFPs are non-toxic proteins isolated from living organisms. Unlike KHIs, antifreeze proteins are biodegradable.[6] A synthetic dye called Safronin-O, has also displayed the ability to bind to and inhibit ice crystals via a similar mechanism as AFPs, as supramolecular assemblies of the dye possess a similar structure to the proteins.[12]

1.3 THF hydrates as a model crystal

In my research, Tetrahydrofuran (THF) hydrates were used as a model for methane hydrates, in order to investigate the interaction between inhibitors and hydrate surfaces. The choice of THF hydrates as a model in the laboratory is due to their ability to form at atmospheric pressure and temperatures just above 0 °C, instead of the extreme conditions, such as high pressure, required by methane hydrates.[4] Additionally, these hydrates are preferable to work with because THF is completely miscible with water, whereas forming hydrates on the liquid-gas interface with methane would be more difficult.[13] The stoichiometric ratio for THF hydrates is documented as 17 molecules of water for every molecule of THF, which is the ratio used in our laboratory to create solutions.[14,15]

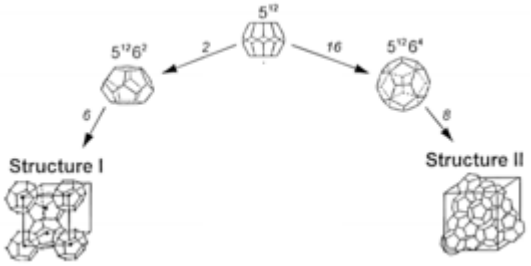


Figure 3. Two most common hydrate structure. Image adapted from reference 1.

Methane hydrates and THF hydrates differ in their guest molecules, one being a gas and the other a liquid. These crystals also vary by their structures, because the structure of a hydrate differs by its guest size. Methane hydrates form structure I (SI), a basic unit of 12 hexagonal faces formed by water molecules (Fig 3). This type of hydrate normally hosts small, natural gasses, such as methane, ethane, and carbon dioxide, and is found in deep oceans. On the other hand, THF, which is much larger than methane, forms structure II hydrates (SII). Other molecules which lend to structure II hydrates are propane and isobutane.[1,2] The shape and structure of the hydrates change due to the difference in cavity sizes required to host variously sized molecules. The cavity size lends to whether or not a guest molecule can fit inside, and the guest molecule can also lend to the stability of the

cavity.[1] The cage and guest molecule do not require a specific size ratio, rather a certain molecular size range can only fit in one type of hydrate structure. Additionally, each cavity can only host a single guest molecule, and more than one guest molecule enclathrated in a water cage is not considered a normal occurance.[2]

Another difference between hydrocarbon hydrates and THF hydrates, which can possibly cause a difference in their structures and surface properties, are the guest molecules' differences in polarity.[7] THF more readily interacts with water, as it is less hydrophobic than hydrocarbons due to the presence of oxygen in its ring (Fig. 4). On the other hand, hydrocarbons are completely nonpolar and do not interact with water. Since the cage is made out of water molecules, if the guest molecule interacts and forms hydrogen bonds, it can affect crystal surface properties.

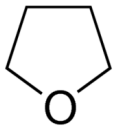


Figure 4. Structure of tetrahydrofuran (THF).

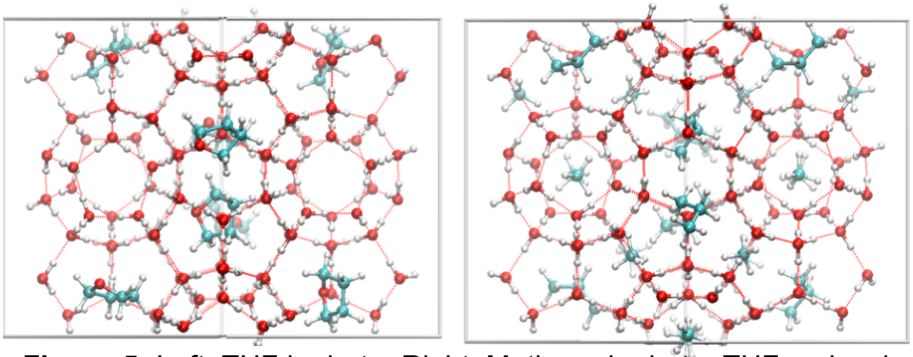


Figure 5. Left: THF hydrate. Right: Methane hydrate. THF molecules only occupy the larger cavities. Taken from reference 17.

Despite differences in hydrate structures and properties of guest molecules described above, experimenting with hydrates of differing guest molecules is the current method for studying inhibitors, and THF hydrates have been used as a model in many studies in order to draw conclusions on the properties of natural gas hydrates[4,8,13–17]. Importantly, it has been

recently displayed that THF hydrates possess similar mechanical and vibrational properties as natural gas hydrates, despite differences between the crystals on a molecular level (Fig. 5).[17] Therefore, the preferential use of THF hydrates in laboratories due to their growth in atmospheric pressure and non-extreme temperatures is further justified, minimizing the need for expensive, high-pressure equipment. The study computed properties of THF hydrates and found that they fell within the literature values of hydrocarbon hydrates.[17] For example, the elasticity, or resistance to compression, of THF hydrates was similar to that of hydrocarbon hydrates including methane, ethane, propane, and isobutane. This finding reasons that the number of hydrogen bonds per unit volume between water molecules, or hydrogen bond density, is similar between these differently structured crystals. Similar values for the hydrate lattices were found between the crystals as well, which has important implications on the ability of inhibitors to bind. Additionally, the average angle between 3 oxygen atoms of water molecules on the hydrate surface were found to be identical between the different hydrates. While these data are all promising, there was one property which might divide THF hydrates and hydrocarbon hydrates. The hydrogen bonding between guest and host molecules was not so simple to compute, as fluctuation in temperature could greatly influence these values. This study was computed at low temperatures, where those hydrogen bonds are less likely to occur, and concluded that THF hydrates make accurate proxies. However the question of whether or not THF hydrates can be accurately used as models for hydrocarbon hydrates at higher temperature still holds, despite this encouraging study, due to the potential of guest-host hydrogen bonding.

1.4 Research objectives

One goal of my research is to further understand how kinetic hydrate inhibitors interact with the crystal surface. More specifically, I investigated the influence of molecular weight on the inhibiting capacity of PVP. I addressed this question by comparing kinetic hydrate inhibitors, and exploring their chemical interactions with the hydrate surface, to further unravel the mechanism of inhibition on THF hydrates. Another objective was to develop a new method of growing THF hydrates. Rather than grow the crystals macroscopically, in the Drori laboratory, we have formulated a methodology in which microscopic THF hydrates can be grown under the microscope, which enables quantitative measurements of inhibition activity. The Drori laboratory has been a pioneer in creating this methodology of analyzing THF hydrates, and it is not performed in any other laboratory so far.

2. Hydrate surface interactions of inhibitors

2.1 Antifreeze protein inhibition mechanism and the Gibbs-Thomson Effect

Studying and understanding the mechanism of inhibition for various types of inhibitors is important, as it can lead to the production of inhibitors which mimic their properties. Antifreeze proteins are well studied inhibitors, and their mechanism for the inhibition of ice growth is the most understood amongst inhibitors. These proteins decrease the freezing point of ice crystals, meaning that a lower temperature is required to grow, in a non-colligative way.[18] Thereby, the presence of solute in the solution, which would normally cause a freezing point depression due to the disorganization of water molecules, is not the main determinant of a freezing point depression here. Antifreeze proteins cause a freezing point depression, however, they also induce a separation

between the melting temperature and the freezing temperature, a distance in degrees coined the thermal hysteresis gap (TH). This activity is a product of the proteins' direct interaction with the crystal surface, and not their presence in solution, which is the mechanism of salts and solutes in decreasing the freezing point, as with THIs. Additionally, AFPs present a saturation level where activity plateaus, however salts display a linear relationship between increased concentration and freezing point depression.[18] This feature provides the active restriction of antifreeze proteins, as there is a limit to the surface of a crystal, while the ability to disrupt individual water molecules is more available.

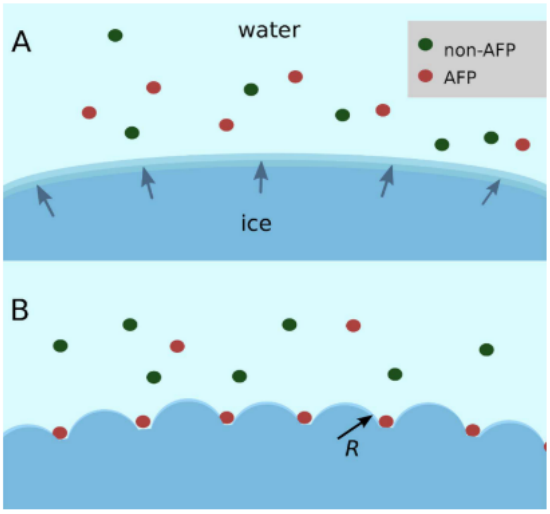


Figure 6. (A) Ice crystals growing at a normal freezing point before the adsoprtion of antifreeze proteins. (B) local curvature caused by bound AFPs decreases the freezing poin past a critical radius. Taken from Reference 19.

Adsorption of antifreeze proteins onto crystal surfaces results in crystal growth inhibition, which is measured using thermal hysteresis activity. This inhibition is explained by the Gibbs-Thomson (Kelvin) Effect. When an antifreeze protein adsorbs to the crystal surface, it inhibits ice growth where it is bound, and ice can continue to grow nearby. This causes a localized micro-curvature of the surface surrounding the bound AFPs, lowering the probability of growth for the ice crystals and decreasing the freezing point (Fig. 6).[4,18,19] When the crystal surface experiences curvature, ice growth is less likely to occur because the surface has access to fewer water molecules from the

surrounding solution. Therefore, the water molecules are less likely to form hydrogen bonds with the ice phase, and the crystal won't grow. A crystal with a large surface and low curvature, as seen in Fig. 6A, is able to grow at normal freezing temperature. However, a crystal with a large surface and high curvature due to the subdivision of the surface by inhibitors, as seen in Fig. 6B, is unable to grow and reaches equilibrium with the solution at a much lower temperature.[19] Therefore, the freezing point is depressed, and the thermal hysteresis is inversely proportional to the radius of a spherical crystal, as described in the following equation:[18]

$$\Delta T = T_o - T = (2\Omega\gamma T_o) / (\rho_{min}\Delta H_o)$$

Here, $T_o - T$ is the difference between the freezing temperature and equilibrium melting temperature, or TH gap, and ρ_{min} is the radius of the added spherical surface. Since the radius is located in the denominator, it is inversely proportional to TH, and a smaller radius will cause a greater gap between freezing and melting temperatures. Other terms in the equation include Ω, which is the molar volume of ice, γ, the isotropic surface energy, and ΔH_o, or latent heat of fusion.[18]

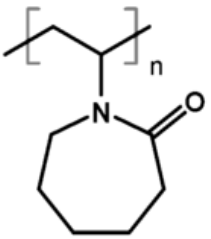
The Gibbs-Thomson Effect applies to antifreeze proteins on inhibitting ice growth, as described, however when a TH gap occurs as a result of inhibition for other crystals, such as hydrates, or other types of inhibitors interacting with the crystal surface, the activity is also best been explained by the phenomemon of localized surface curvature. Therefore, when AFPs display TH activity on hydrates as well as ice crystals, it can be assumed that they interact via the mechanism of the Gibbs-Thomson Effect. Kinetic hydrate inhibitors like PVP and PVCap also exhibit thermal hysteresis activity, rather than solely causing a decrease in freezing temperature, as they interact directly

with hydrate surfaces. This decrease in freezing temperature is also called 'supercooling,' as the temperature of the liquid is cooled to a point where solidification is expected, but is not observed.

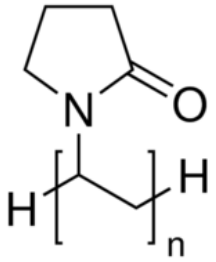
2.2 Hydrate surface interactions and properties of PVP and PVCap

PVCap and PVP are water soluble kinetic hydrate inhibitors, which are currently used in industry to prevent the growth and accumulation of hydrates in pipelines. In the presence of PVP and PVCap inhibitors, the hydrates formed are elongated and shaped as octahedra.[5]

Many factors affect the ability of these polymers to inhibit hydrates. The first is its prevention of hydrate formation. In PVP and PVCap, the oxygen substituent on the cyclic amide can form a hydrogen bond with the hydrogens of free water molecules meant to encage a guest molecule.[7] Due to this interaction, the inhibitors are able to disrupt the organization of water molecules and the guest molecule, which prevents initial nucleation. Once nucleation eventually occurs, it begins in the form of hydrate nuclei, which are microscopic hydrates, with sizes in the order of tens of angstroms.[5] Interestingly, antifreeze proteins also prevent the formation of hydrate nuclei, but only begin to inhibit ice crystals once their nuclei have formed.[6]



Polyvinylcaprolactam (PVCap)



Polyvinylpyrrolidone (PVP)

The next level of activity from these KHIs is their inhibition of formed hydrate nuclei. Using molecular dynamics simulations, the mechanism of this hydrate surface interaction of kinetic hydrate inhibitors can be further explained. PVCap and PVP have a similar charge distribution between the oxygen and a carbon on their rings. This charge separation mimics the one found in water molecules located on the surface of the hydrate, forming a binding site for the inhibitors and allowing them to form strong hydrogen bonds.[5] The binding strength of kinetic hydrate inhibitors correlates to its activity levels. Simulations were also able to predict where on the hydrate surface these kinetic inhibitors were bound, specifically for structure II hydrates of THF. PVP was shown to occupy the central cages, and PVCap displayed the ability to bind to the empty cages of THF hydrates.[4] Additionally, both kinetic hydrate inhibitors were experimentally shown to adsorb irreversibly to the crystal surfaces of SI and SII hydrates.[4,14] These binding positions would effectively reduce the probability for gas incorporation in the cages of structure II hydrates, and prevent further growth.[4] Looking back at figure 5, since the size of THF is much larger than methane molecules, it only occupies the larger cages. Therefore the ability for PVP to bind to the central cages and PVCap to the larger, empty cages, might not be consistent with methane hydrates, which are structure I hydrates with smaller cages.[4] However, since methane occupies all the cages in the hydrates, the binding of PVP and PVCap to the cages is still possible, and it is known that PVP inhibits methane hydrates.[5,6] PVP and PVCap have also been shown to inhibit structure I hydrates of ethylene oxide (EO), which is applicable to the structure of methane hydrates.[14] An explanation as to why the binding of these kinetic inhibitors to the hydrate surface causes

an inhibition of growth is that their binding prevents the crystal from growing in between the polymer strands, similar to the Gibbs-Thomson Effect previously mentioned.[14]

When comparing the inhibition activity of PVP and PVCap, the monomer size plays an important role[5,6,14]. Specifically, the size of the PVCap ring has been shown to contribute to the binding of the hydrate surface more than PVP's smaller ring. This is because PVCap's larger ring has a more limited molecular motion, meaning it is more restricted, when bound to the hydrate binding site, reflecting stronger binding, whereas PVP's smaller ring does not cause the same stabilization of the cavity.[5,14] Additionally, if more space is occupied by an inhibitor at the hydrate surface, there will be further prevention of the association of guest molecules, and thereby inhibition of hydrate growth.[5,7] It has also been hypothesized that the rings of these polymer inhibitors bind to unfinished cavities, and act as pseudo-guest molecules, with favorable interactions due to the hydrogen bonding of the carbonyl group.[14] Based on this mechanism of inhibition, the length and bulk of the polymers are important factors which contribute to their inhibition activity, and importantly, display that properties other than hydrogen bonding are vital to increased inhibition of hydrates. PVPCap is also able to form multilayered polymer adsorptions on the hydrate surface, taking up more space and increasing inhibition.[7] These theories follow the conclusion realized by experiments that PVCap has a greater

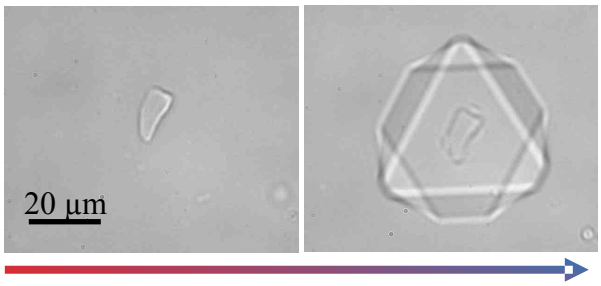


Figure 7. A THF Hydrate before (left) and after (right) burst. As temperature decreases, the crystal bursts with the original crystal still visible.

inhibition activity than PVP, greatly due to its difference in ring size and polymeric folding.[5,7,9,14]

3. THF hydrates in the Drori laboratory

3.1 Experimental procedure

In the Drori laboratory, we measure the TH activity of various inhibitors at different concentrations. The greater the distance between the melting point and freezing point of a crystal, the more active the inhibitor. Meaning, an inhibitor with a large thermal hysteresis gap will prevent the crystal from freezing, or growing, until much lower temperatures, as compared to an inhibitor with low TH activity. Since we analyze single crystals manipulated with discrete and controlled temperature fluctuations, the melting point of a crystal can be documented as the temperature reached at which the crystal has just stopped melting. This point is achieved by increasing the temperature from a freezing crystal, to one in which the crystal is melting very slowly. Next, the temperature is decreased in 0.02°C increments until the temperature is reached where the crystal just stops melting, and does not grow either. Finally, the temperature is decreased at a rate of -0.05°C every 4 seconds until a rapid growth, or burst, of the crystal is observed (Fig. 7). This is considered the freezing temperature of the crystal. The reason for this rapid burst is due to the supercooled solution, at a temperature well below its freezing point. Therefore, once the crystal is able to grow, it does so relatively fast. The value for TH is found by calculating the absolute value of the difference between the burst point of the crystal and its melting point.

3.2 Measured activity of PVP inhibitors on THF hydrates

In my research, I tested the TH activity of PVP on THF hydrates in various molecular weights, and hypothesized the larger (by molecular weight) polymers to have increased activity, due to the relationship in size and inhibition activity of the very similarly structured inhibitors, PVP and PVCap. The idea that larger molecules have increased TH activity is also supported by previous studies. It has been displayed in antifreeze glycoproteins (AFGPs), that the longer the inhibitor, the higher the TH activity.[20] Another study included PVP inhibitors, and their inhibition of methane hydrates using pressure fluctuation in a rocking cell.[6] The authors found that PVP10, weighing 10kDa, displayed increased methane hydrate nucleation inhibition performance as compared to PVP40, the polymer of higher molecular weight. The smaller polymer was more able to inhibit the hydrate from nucleating originally, because it can more easily interact with water molecules prior to their organization[6]. As previously mentioned, hydrogen bonding is important when it comes to delaying hydrate nucleation.[5,7] Oppositely, PVP40 displayed increased inhibition of methane hydrate growth as compared to PVP10.[6] This points to a property other than hydrogen bonding as integral to the inhibition of the hydrate surface, since PVP40 is not as capable as PVP10 to hydrogen bond, although it does display increased inhibition of the formed crystal. Size and hydrophobic repulsion of water, as displayed in antifreeze proteins, are prime candidates.[4,6,10] Additionally, since PVP40 has a higher molecular weight than PVP10, it seems as though size and hydrophobicity are involved in the interaction of the inhibitor at the hydrate surface.

In our study, single crystals of THF hydrates were observed in the presence of PVP10, PVP40 and PVP360 and their TH was measured. Similar results were originally

expected. However, a trend of increased molecular weight following increased TH activity (or crystal growth inhibition) of the THF and methane hydrates may hint to similar mechanisms at the surfaces of both crystals.

Figure 8 presents the TH activity vs. the squared root of the polymer concentration, which was used to linearize the plots. A clear increase in TH activity was observed with increased concentration, for all three polymers of PVP. Additionally, PVP40 and PVP360 display overall higher activities as compared to PVP10, the smaller polymer. However, PVP360 and PVP40 have almost identical activity, implying that while TH activity increases with increased molecular weight of PVP, it plateaus at and

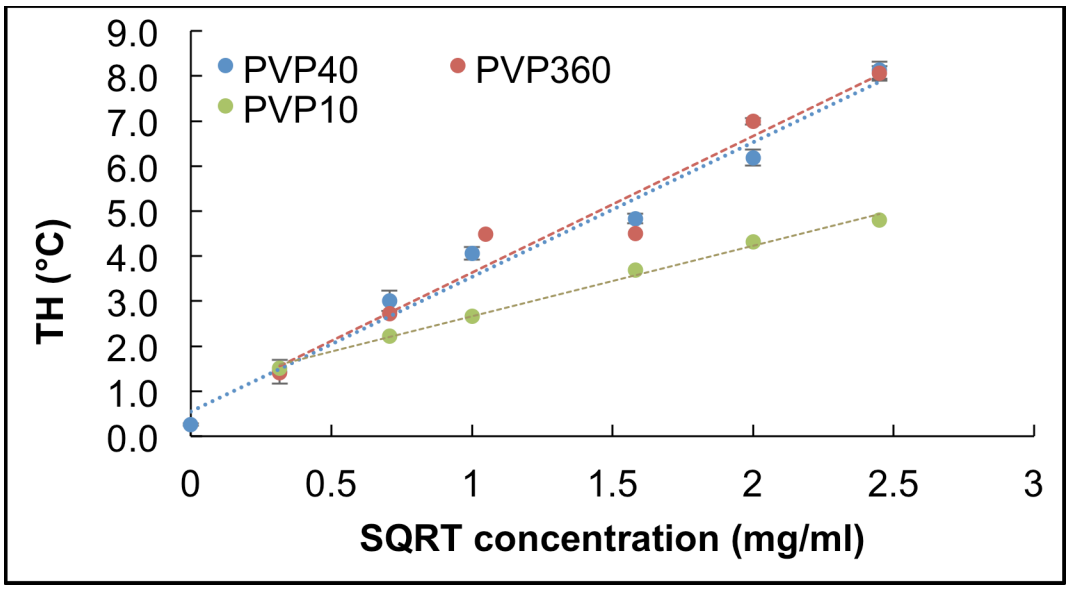
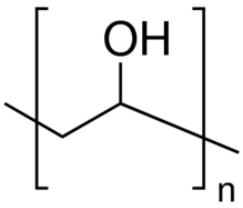


Figure 8. Graph of thermal hysteresis activity as a function of the square root of concentration, for three different polymers of the kinetic hydrate inhibitor, PVP. An increase in activity with increase molecular weight of the polymer was observed.

after PVP40. Additionally, this effect of increased activity with larger polymers only occurs at higher concentrations, namely above 1 mg/ml.

3.3 TH activity of PVA on THF hydrates

Polyvinyl Alcohol (PVA) is a polymer which is soluble in water. It contains a characteristic hydroxyl group, and when dissolved in water, folds into a helical structure with multiple strands. This molecule would be useful to compare to our PVP results, since it lacks a hydrophobic ring, and interacts with the hydrate surface solely via hydrogen bonding.



Polyvinyl Alcohol

Studies have shown that PVA contains ice growth inhibiting activity, as well as the ability to inhibit ice recrystallization. Additionally, it has also been found that PVA can weaken the adhesion force of THF hydrates.[8] However, it seems that PVA is the least active kinetic hydrate inhibitor. According to one study, PVA was the least active inhibitor among antifreeze proteins as well. These researchers found that AFP I was the most effective at inhibiting the growth of THF hydrates, based on the resulting degree of supercooling, followed by PVP, AFPIII, and finally PVA (Fig. 9).[8] In fact, some researchers came to the conclusion that while PVA displays ice crystal growth inhibition, it is not an inhibitor towards THF hydrates, since its performance was similar to that of the hydrate without any additives.[14] These experiments were performed by analysis of the hydrate-solution interface, as mentioned later in the methods section.

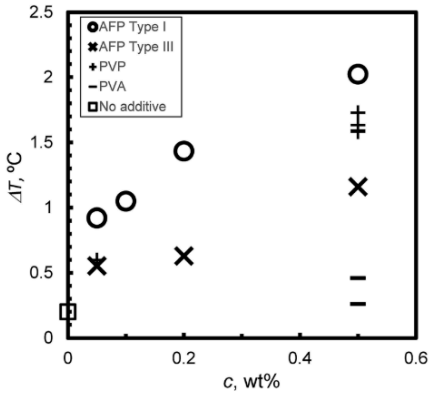


Figure 9. Thermal hysteresis as a function of concentration for various inhibitors. Taken from reference 8.

Data collected from another student in the Drori laboratory, however, displays some amount of TH activity using PVA on THF hydrate surfaces.

Using concentrations of 1-6 mg/ml, TH values around 0.3 - 1.3 degrees were obtained. While this data is still being collected, it is clear that PVA possesses some inhibition properties of THF hydrates, although not nearly as much as PVP, which at a concentration of 6 mg/ml possessed TH values near 8°C for PVP40 and PVP360 and 4.7°C for PVP10 (Fig. 8). This slight inhibition activity can be due to the design of our experiments, which allowed the visualization of single crystals micrometers wide. This technique is much more accurate than analyzing an interface the length of millimeters. This distinction could be the reason why the data for PVA, the straight line data point as seen in figure 9, was so similar to the hydrate without additives, the box on the y axis. Additionally, the concentrations used in our experiments were much higher than those used in the other study.

3.4 Discussion of the mechanism of inhibition of PVP kinetic hydrate inhibitors

Based on the results of my experiments, as well as the findings from other studies, it can be concluded that a property other than hydrogen bonding is important to the mechanism of inhibition of THF hydrates by KHIs. This is because PVA displays much lower activity than PVP. As the least effective inhibitor of hydrate crystals, PVA is made up of the smallest percentage of hydrophobic regions. The mechanism of adsorption of this polymer onto the crystal surface most likely involves hydrogen bonding of the hydroxyl groups. However, other inhibitors such as AFPs, PVP, and PVCap, have greater inhibition activity, and much larger hydrophobic regions, which have proven important to its binding mechanisms.[4–6,10,14,19] Particularly, the hydrophobic rings of PVP and PVPCap are able to bind to the cavities of hydrates to prevent crystal growth and effectively exclude guest molecules.[4,14] Presumably, PVA would not be able to conduct the same

interactions, or at least not to the same degree of efficiency. Therefore, while hydrogen bonding via the carbonyl carbon of PVP and PVCap might play a role in the adsorption of inhibitors onto the crystal surface, it seems to be more of a secondary aid to the rich, non-polar regions, which likely possess a greater contribution to the binding strength and efficacy of hydrate inhibitors.[4,14] However, the greater inhibition activity could also be due to the ability for PVP and PVCap to take up more space on the hydrate surface due to their ring sizes, rather than due to their hydrophobicity, which PVA also lacks. The property of size, and increased space taken up by the inhibitors would contribute to the association of the moelcules with the hydrate cavities, as well as the increased prevention of additional molecules to the hydrate. This conclusion also supports my data above.

In the data I collected, it was clear that increased size of PVP leads to an increase in TH activity. As mentioned, PVP and PVCap function by binding to unfinished cavities, and have displayed increased capacity with size in preventing the accumulation of guest molecule in these cavities.[4,5,7,14] The increase in activity with molecular weight observed may indicate a folding mechanism of PVP polymers of greater length, which enhances their ability to stabilize the cavity, or, to take up more space on the hydrate surface, in order to prevent growth or the addition of guest molecules. By taking up more space, crystal growth is prevented between the polymer strands, and grows in smaller curvatures, increasing TH activity via the Gibbs-Thomson Effect.[14] The fact that these enhancements from molecular weight plateau may imply that, if a folding mechanism is at play, it continues to occur past the size of PVP40, but this effect diminishes at some polymeric size beforehand. Therefore, it would be interesting to see where PVP20 and PVP30 polymers fall along this observed trend.

4. Methodological evolution of hydrate growth in the laboratory

4.1 Changes in morphology as a method for identifying inhibition activity.

There are several ways to investigate the activity of crystal inhibitors. These are measuring the extent of growth inhibition, which in the case of ice and hydrates can be the thermal hysteresis (or TH) gap, as is the current method mentioned in the Drori laboratory, and analyzing changes in morphology of the crystal.

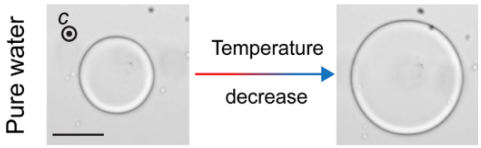


Figure 10. An ice crystal in pure water solution immediately grows spherically upon a decrease in temperature. Taken from reference 12).

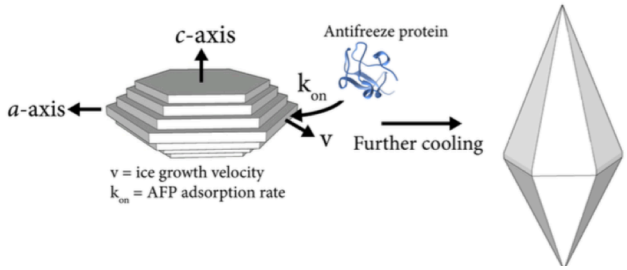


Figure 11. Right: A bipyramidal ice crystal in the presence of AFPs which inhibit the a-axis plane. Left: Step-wise growth of an ice crystal, depicting the binding sites of different antifreeze proteins as well as the kinetic competition between ice growth and antifreeze protein binding.

Morphology changes of a crystal in the presence of inhibitors, suggests that those inhibitors are bound to the surface of the crystal and are preventing growth on a face of the crystal. For example, in the absence of additives, pure ice crystals grow spherically, and without a TH (Fig. 10). However, in the presence of certain antifreeze proteins, the crystals grow in a bipyramidal shape because some of the planes of ice are bound by the inhibitors (Fig. 11). For example, when AFP III inhibits the a-axis, ice can freely grow on the c-axis. Ice growth along the c-axis is then accelerated, and in the Drori laboratory we have observed the rapid growth of this axis when forming the bipyramidal crystal. Other studies have also documented rapid initial growth of crystals in the presence of AFPs.[6] However, each step formed along the c-axis will be smaller than the previous one. Eventually, the plane

will become small enough that the probability of ice growth is diminished significantly. It forms a sharp point, hence the bipyramidal shape. Once this shape is formed, the crystal no longer grows, as the ice growth velocity is less than the adsorption rate of antifreeze proteins. Once the velocity of ice growth wins, the rapid growth is seen.[21] Therefore, a change in morphology can theoretically reflect the activity of an inhibitor. Different AFPs, which bind to other planes of ice, cause different morphological changes to the crystal.

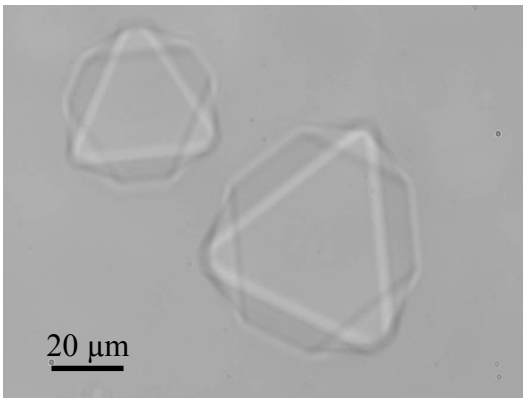


Figure 12. THF hydrate crystals grown in the laboratory, shaped as octahedra. 6 points are visible in this image, with two additional points: one coming out of the page and one into the page.

Unlike ice crystals, THF hydrates are octahedral in shape (Fig. 12). These crystals display thermal hysteresis activity as well as morphological changes in the presence of inhibitors. In fact, measuring the change in morphology of hydrates is a method used in various studies in order to compare the activity levels of different inhibitors of THF hydrates (Fig. 13).[4,8,11,14,15] Additionally, similar to the effect of AFPs on ice, different inhibitors can cause different morphological changes in THF hydrates. For example, from pure melt, an octahedral crystal will grow. However, when exposed to differing inhibitors, an array of morphological changes can occur such as flat, two dimensional hexagonal

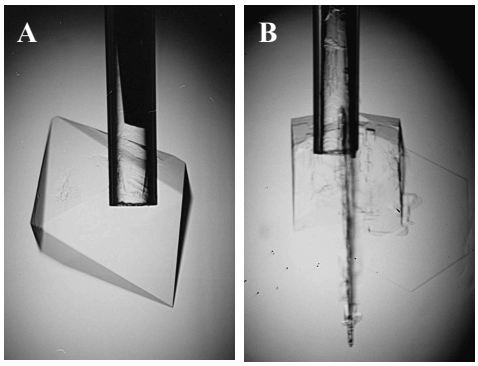


Figure 13. Morphological change in THF hydrates from an octahedral crystal control (A), and 2D hexagonal plates when exposed to the inhibitor PVCap (B). Taken from reference 14.

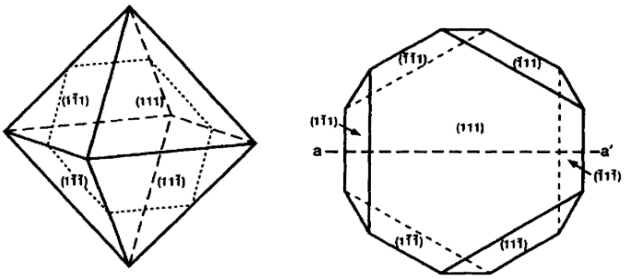


Figure 14. The binding surface, {111}, of octahedral THF hydrate crystals. Image taken from reference 15.

planes, kinks at the surface, and platelike polycrystalline or needle shaped hydrates.[15]

Different morphologies of the hydrate surface can reflect different modes of binding for the inhibitors. These can be differences such as binding to different faces of the crystal, as PVP and PVCap have been shown to bind to the {111} faces of THF hydrates, or displaying distinct mechanisms of inhibition, such as binding to organized water molecules sandwiched between the crystal surface, as has been documented for AFPs (Fig 14).[11,13–15,19] Morphological changes can also be used to predict the activity levels of various inhibitors, based on the degree of morphological change that is caused.[8]

4.2 Growing macroscopic THF hydrates and measuring changes in morphology

The macroscopic analysis of THF hydrates and their morphologies was the first methodology undertaken in the Drori laboratory (Fig. 15). These experiments were done in a beaker which was placed in liquid ice solution, or dry ice, inside of a styrofoam box. A copper cold finger was placed in the solution in order to spur the nucleation of hydrates. Since copper is a good thermal conductor, it becomes colder than the solution in the beaker, creating local supercooling and a surface for nucleation, which will spur the formation of hydrates.[14] Additionally, with the cold finger, crystals can be analyzed and easily removed from the beaker. However, many challenges were faced with these experiments. For example, it proved extremely difficult to control the temperature in the

chosen set up. The styrofoam box (Fig. 15A) was a useful insulation tool, however, it did not maintain a completely stable internal temperature for the experiments. Dry ice was also used to keep temperatures low, but a control over temperature was inevitably impossible, causing the nucleation of hydrates to be either unsuccessful or unpredictable. An additional challenge was the lack of quantitative data for the inhibition of hydrates, as the morphology changes were qualitative. Lastly, we were unable to successfully isolate single crystals of the hydrate to study. Therefore, it was difficult to compare varying concentrations of an inhibitor, or the activities of different inhibitors.

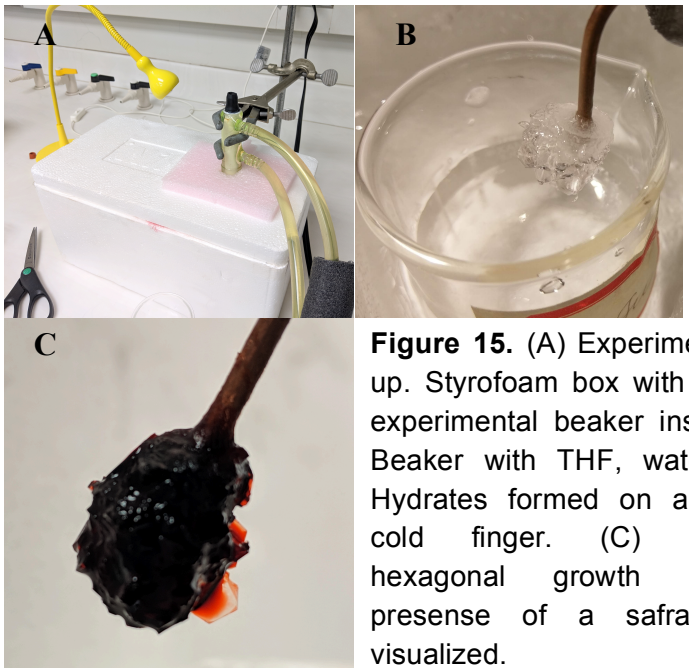


Figure 15. (A) Experimental set up. Styrofoam box with ice and experimental beaker inside. (B) Beaker with THF, water melt. Hydrates formed on a copper cold finger. (C) Platelike hexagonal growth in the presense of a safranine is visualized.

One solution to the lack of quantitative data was to work with more microscopic samples. In one study, the solid-liquid interface of THF hydrates was analyzed for morphological changes at different concentrations of an antifreeze protein inhibitor (Fig. 16).[8] At low concentrations, hexagonal plate crystals were analyzed, which became continuously smaller with increased concentration of the inhibitor, until finally, flat crystals were observed. Therefore a decreased shape of crystals correlated with increased

inhibition. Through analyzing the distance grown and shape change of the interface of THF hydrates and solution by manipulating hot and cold blocks for varying amount of time, the authors were able to quantify the activity of various inhibitors.[8] However, visualizing single crystals would provide elevated accuracy and quantitative data with the ability to calculate thermal hysteresis.

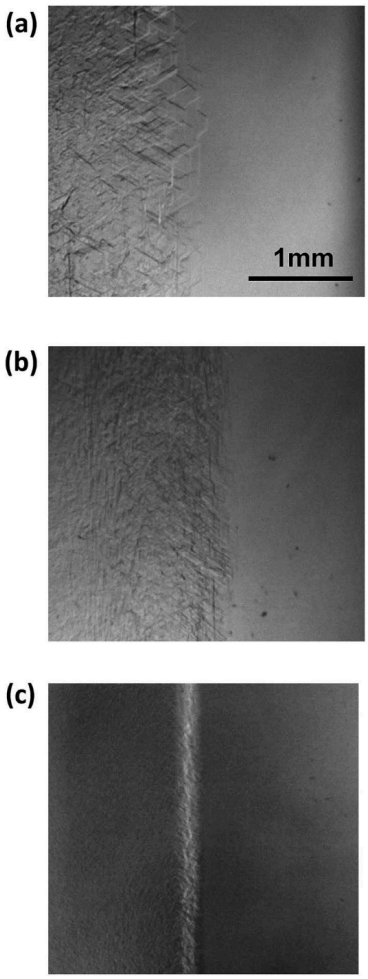


Figure 16. Growth interface of THF hydrates in the presence of AFP I. (a) 0.05 wt%, (b) 0.2 wt%, (c) 0.5 wt%. Taken from reference 8.

4.3 Growing microscopic THF hydrates in microfluidic devices

In the Drori laboratory, rather than observe the hydrate interface, a microscopic observation of single hydrate crystals using PDMS microfluidic devices was the next method in order to qualitatively and quantitatively measure the activity of hydrate inhibitors. PDMS (polydimethylsiloxane) is a transparent, inexpensive, and easy to mold, silicon-based polymer. It is often used in microfluidic devices for its biocompatibility and clarity in viewing microchannels. This microfluidic device was equipped with a main chamber, where the crystals grow, and tunnels leading to inlets and an outlet (Fig. 17). This device is placed on top of a temperature controlled copper plate, which influences the temperature of the solution in the chamber. A thermistor, or resistance thermometer, is placed between the PDMS device and copper plate in order to read the temperature of the solution being

manipulated. Additionally, a copper wire can be punched through the PDMS and inserted into the chamber in order to spur nucleation at that site. Since the copper wire is actually colder than the solution, the crystals grow at a slightly lower temperature than the one read by the thermistor. Inlet and outlet tubes are then added into the microfluidic device, and the former are filled with the desired solutions. For example, one may have aqueous THF solution, while the other will be concentrated with the inhibitor in question. Using this device with microscopic channels is helpful in that single crystals can be isolated via temperature manipulations, and the fluids surrounding the crystals can be exchanged by flushing the chamber with solutions from the inlets. This technique can allow for the study of inhibitors introduced to the surface of pre-existing hydrates in order to further understand the molecular interactions taking place. For example, these devices are used in order to calculate the adsorption rate of AFPs onto the ice surface using a green fluorescent protein tag, as well as the plane binding affinities of the inhibitors.[22] Additionally, less volume of the sample is required in order to conduct experiments, and liquid compositions can be greatly controlled with microfluidic devices.

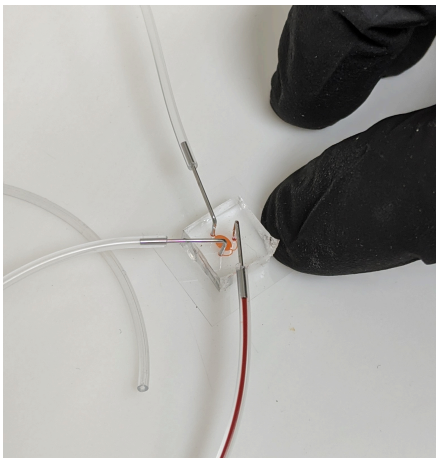


Figure 17. A microfluidic device used in the Drori laboratory, fashioned with 2 inlets, 1 outlet, and a main chamber.

Figure 18. Microscopic view of the chamber in a microfluidic device. The black mass is the shadow caused by the copper wire. Ice is observed around the copper wire, which is about 3 mm in diameter

While the microfluidic device was successful in that individual crystals were able to form on the copper wire, it was difficult to visualize due to a shadow caused by the cold finger (Fig. 18). The cold finger was also useful because it prevented the crystals from melting in the chamber during the flow of solution.[22] Surprisingly, when the cold finger was removed, we were still able to nucleate hydrate crystals. However, because the inlets were colder than the middle of the chamber, the crystals were either only isolated in the tunnels which were hard to visualize, or those crystals would interfere with an analysis of a crystal in the chamber.

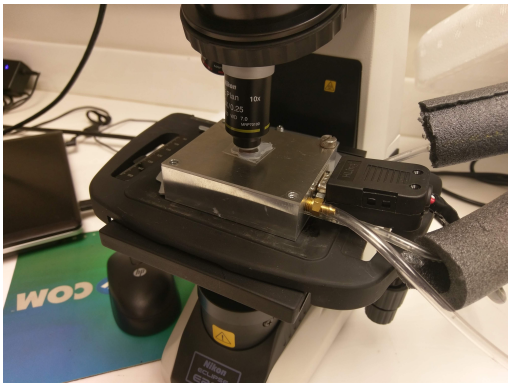


Figure 19. Nanoliter osmometer in the laboratory, featuring a temperature controlled box, copper plate, and circulating water.

4.4 Growing THF hydrates under the microscope

The next and final method for the growth and study of THF hydrate crystals was utilizing a nanoliter osmometer, which is commonly used to measure the thermal hysteresis of inhibitors on ice crystals (Fig. 19).[6,22,23] The reason why this method was not approached from the start was because it was not clear whether or not it was possible to grow THF hydrates in the osmometer, or without a cold finger, as it is for ice crystals. And even if it were possible, it was unclear if the process could be consistently replicated. However, we were able to formulate a methodology which enabled us to grow THF hydrates successfully in water and inhibitor solutions. The nanoliter osmometer is equipped with a temperature-controlled copper plate, and a water circulator which enables energy to transfer so that the temperature can decrease. On top of the copper plate we place a sapphire disc, with a drop of oil on top. Around 2-3 µL of the sample is

injected into the oil, and a glass cover slip is placed on top at a 45° angle. The sample is now a small drop floating inside of oil, so that evaporation is not a concern. Once set up inside the temperature controlled box, the temperature is decreased to around -30°C, or until the entire sample freezes over. The temperature is then increased to near -6°C, as to compensate for any overshoot in temperature fluctuations. Next, the temperature is increased above the melting point of water (0°C), usually to around 3°C, so that the entire sample is void of ice crystals. Since THF hydrates have a higher melting point than ice, at 4°C, any crystals left at this point can be considered hydrates. Usually at this point there is a thin sheet of hydrate over the sample. By successively increasing and decreasing the temperature, larger hydrate crystals will nucleate in the sample (Fig. 20). Finally, the temperature can be increased to cause the smaller crystals to melt away, and then decreased again to enlarge the ones left over. This process is repeated until a single crystal is isolated. Once there is a single crystal, the melting point can be measured, and the TH measurement can begin, as previously described. A hydrate crystal burst looks different than that of an ice crystal (Fig. 7). An ice crystal which is bipyramidal in shape will burst from the tip, or the c axis, in a needle-like extension. Hydrates on the other hand burst into an octahedral shape, growing around the original crystal so that it is still visible at its core.

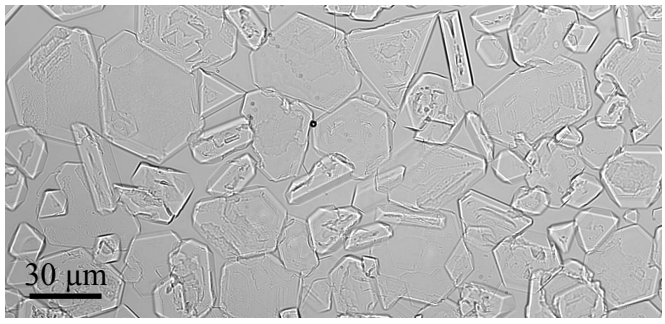


Figure 20. A shower of hydrate crystals covering the sample at 10x objective lens.

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