

Lipid modifications in Archaea

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Contents

1	Introduction	3
2	Short term response and long term strategy	5
3	Hydroxylation	7
4	Saturation	8
5	Extended tails	9
6	Macrocyclic structures	11
7	Cyclopentane and cyclohexane rings	12
8	GDGTs	14
9	Glycosylated headgroups	15
10	Anionic headgroups	16
11	Conclusions	17
12	References	18

1 Introduction

Archaea form a curious branch in the tree of life: only identified as a separate domain of life in 1977 by Woese et al., this group of organisms were found to form a completely separate lineage from either bacteria or eukaryotes through investigation of their ribosomal RNA [1, 2]. Although it was ribosomal RNA that turned out to be the deciding factor for separating Archaea from the rest of the tree of life, it is far from their only atypical feature. Closer examination reveals a rich set of puzzling differences, many of which are to be found in their truly unique membranes. Whereas all bacterial and eukaryotic membranes are primarily composed of fatty acids linked with an ester bond to a sn-G1P backbone, archaea use branched isoprenoid chains linked with a much stronger ether bond to a sn-G3P backbone [3]. Besides the aforementioned characteristics found in all Archaeal lipids, Archaea also show a great variety of unusual lipids and lipid modifications, whose exact physio-chemical function often remains yet to be fully elucidated.

Besides these isoprenoid based core lipids, often called archaeol (AR), the first and most striking modification found in many archaeal membranes is the presence of caldarchaeol, more commonly referred to as glycerol dialkyl glycerol tetraethers (GDGT), a long membrane spanning tetraether resembling two normal archaeol lipids glued together at their tails. It is only within the structure of these long C40 chains that another set of remarkable modifications can be found: the presence of 1-8 cyclopentane rings, sometimes also accompanied by a cyclohexane ring, in which case it has 4 cyclopentane rings and is called cre-narchaeol. Other noteworthy modifications to archaeal lipids are the addition of hydroxyl groups, unsaturated bonds, and the addition of a link between two adjacent tails resulting in a macrocyclic structure, all of which are found in both archaeol and GDGT. Last but not least is the presence of extended C25 tails in archaeol as compared to the normal C20 tails. Archaea show a host of strange lipids exhibiting a combination of these modifications [4].

For a long time most of these strange membrane modifications, especially GDGT and its ring moieties, were thought to be adaptations to the extreme conditions where most Archaea up until then were found [5]. The extremely stable and rigid chemical structure of GDGT and its rings were thought to have evolved to manage membrane stability in extreme heat or acidity [6]. This changed with the discovery of a sizable population of mesophilic ocean dwelling archaea. Within this population initially called the Marine Groups (MGI-III), it was discovered that Marine group I, later identified as Thaumarchaeota ('thauma' fittingly being Greek for 'miracle') features membranes rich in GDGT and ring moieties previously thought to be exclusive to extremophile conditions [7, 8]. With these and other mesophilic occurrences of Archaea, the previously clear cut roles of many of these modifications became more ambiguous.

The question remains what kind of physio-chemical properties these modifications have exactly and what different roles they play in the functioning of archaeal membranes. Although research into their exact physio-chemical properties is often lacking, a lot of information about their function can be gleaned

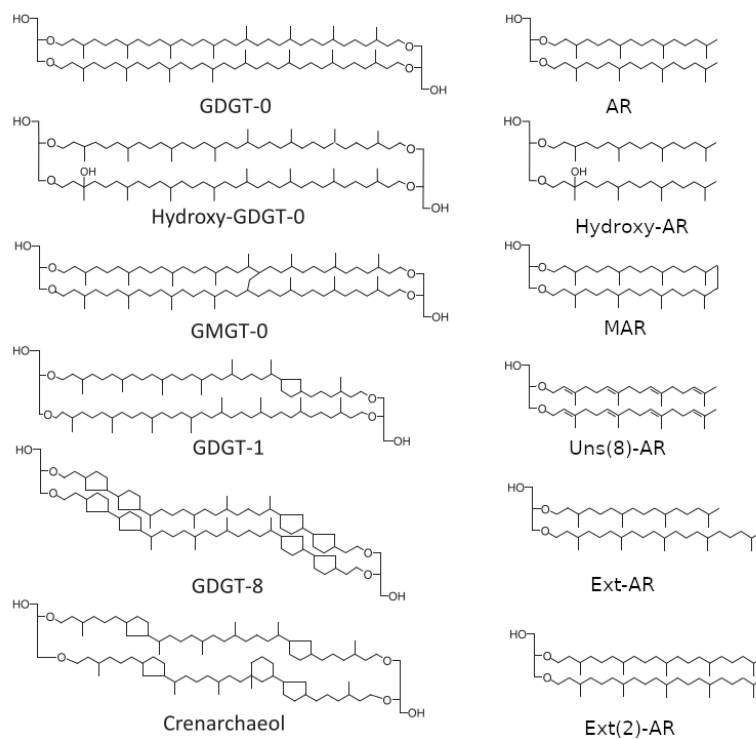


Figure 1: lipid modifications discussed in this paper

from combining the available research with information regarding the phylogeny and survival strategies adopted by the Archaea expressing these modifications. In fact, comparing the specific survival strategies of the different Archaea often gives useful hints as to why certain modifications are used in a way that is hard to deduce from just investigating the physio-chemical properties of the modifications alone. This will then be the aim of this paper, to try and uncover the role of each adaptation through investigation of both their direct physio-chemical properties and the specific survival strategy of the organisms where these adaptations are found. This will hopefully provide a clue both as to what each modification does and why certain phyla of Archaea use certain modifications while others do not.

2 Short term response and long term strategy

Besides direct investigation of the physio-chemical properties of these modifications using either in vitro experiments or molecular dynamics (MD), there are roughly two ways you can look at the function of the modification in the organism itself: Either you look at the short term membrane adaptations of the organism when confronted with different external conditions, or you look at how the overall membrane composition plays into the more long term survival strategy of each particular species and how in their phylogenetic history they came to acquire this strategy [9]. Each offers slightly different yet complementary insights into the functioning of these modifications.

The first method is a fairly intuitive and straightforward approach to investigate the role and properties of lipid modifications. Looking at up- or downregulation of certain modifications when conditions are changed tells you rather directly if the organism benefits or suffers from the presence of these modifications under these new conditions since it is the organism itself that actively changes its membrane as a reaction to the external change. Much of these short term adaptations are likely part of a homeoviscous response, where the organism adapts its membrane to maintain the same membrane properties in order to keep its metabolism and reproduction functioning optimally [10]. These up or downregulations will therefore tell you how each modification is used to counteract the external change.

Although this approach is very fruitful, it often fails to explain much of the more varied use of these modifications and why different membrane compositions are used by different branches of Archaea to begin with. Sometimes certain useful modifications are used by some organisms and not by others even though conditions are similar. Sometimes adaptations that only seem useful for certain extreme conditions are also found outside of this range. To understand these divergences from the expected patterns, it is also important to look at the long term survival strategies of these organism and the phylogenetic history of how they got these traits.

The two main phyla of interest to us are the Euryarcheota and the Proteoarchaeota (often called Crenarchaeota in older literature, which is now considered a subbranch of the Proteoarchaeota). For the Proteoarchaeota, although many other exist, two are most widespread and well researched, the Crenarchaeota and the Thaumarchaeota. The Crenarchaeota are the original namesake of the phylum and exclusively live in extreme conditions, usually hyperthermophilic and hyperacidophilic. The Thaumarchaeota were discovered much later and as previously mentioned, live in more mesophilic marine environments.

Regarding the phylum of Euryarcheota, the research is much more spread out over the different groups. A large portion of the Euryarcheota have a methane generating anaerobic metabolism, like Methanococci, Methanobacteria, Methanopyri, Methanocellales, Methanosarcinales, and Methanomicrobiales. Although they are spread throughout various branches of the Euryarcheota, the text will refer to these more generically as methanogens. Besides these, one particular group of interest are the Halobacteria, a group closely

related to the methanogens that nevertheless adapted a completely different survival strategy. Despite their close relation to methanogens, Halobacteria are aerobic, non-methanogenic, halophilic Archaea that feature unique membrane adaptations to cope with hypersaline conditions.

As previously mentioned, a lot can be learned from why certain branches of Archaea use certain modifications while others do not. In order to compare the different uses and functions of each modification, it is easiest to start with the more niche and specific adaptations in order to compare these with the more common adaptations that occur in a wider, harder to pin down range of conditions. We therefore start with the more specific use of hydroxylation, and work towards the very common but somewhat enigmatic use of GDGT. Finally, besides these modifications to the lipid core, we give a short overview of some modifications to the polar headgroups. of Archaea.

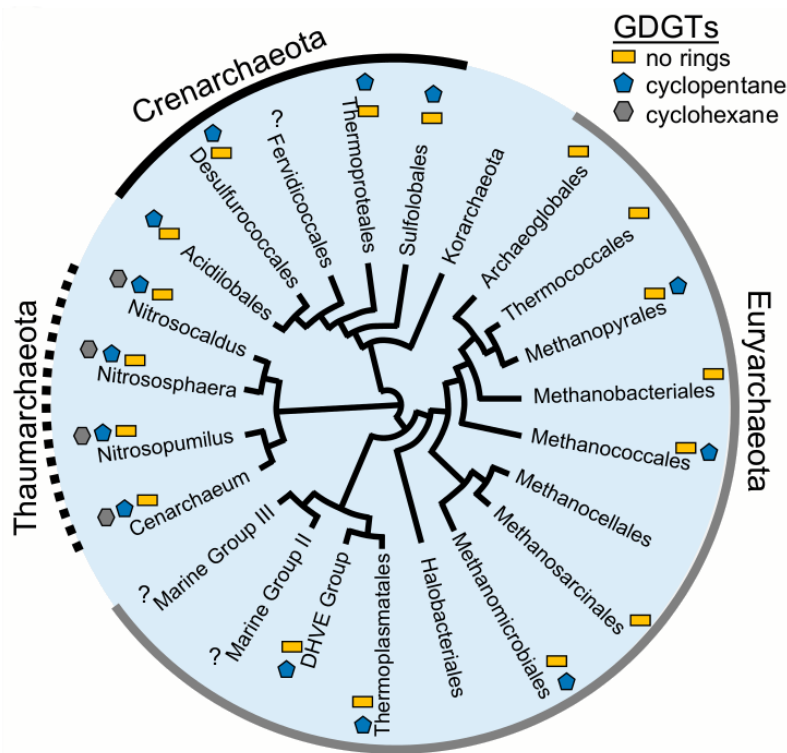


Figure 2: phylogenetic tree of relevant Archaea showing the presence of GDGT and cyclopentane and -hexane rings. Figure taken from Pearson [11]

3 Hydroxylation

Hydroxylation of archaeal lipids always occurs on 3rd carbon of the lipid chain, relatively near the headgroup [12]. Hydroxylation has been reported in both archaeol and GDGTs. In archaeol hydroxylation only occurs in euryarcheotic methanogens and is considered characteristic of Methanosarcinales [13]. Hydroxylated GDGTs on the other hand were originally found in ocean sediments and mostly seem to come from the Thaumarchaeota producing these sediments [14]. They are not exclusive to Thaumarchaeota however, and hydroxylated GDGTs have been found present both Proteoarchaeota and Euryarchaeota [14, 15].

As an adaptation, hydroxylation is strongly associated to lower temperatures, although its exclusivity as a cold response is not confirmed. Little research has been done regarding hydroxylation as a short term response to dropping temperatures in Archaea, but it is known that some bacteria dynamically increase hydroxylation of their membranes to deal with cold temperatures, so similar mechanisms might be expected in Archaea [16, 17]. Its role is more apparent as a long term species-specific trend. Hydroxylated archaeol is generally characteristic of Euryarcheotic methanogens living in colder environments and its occurrence in oceanic waters is exclusive to colder, deeper anoxic regions [18, 19].

The same is true for hydroxylated GDGT which become increasingly more common near temperate to polar oceans, further confirming its function as an adaptation against cold environments [20]. Interestingly, it is still possible for 1-2 cyclopentane rings to occur in these hydroxylated GDGTs, which are adaptations usually associated with increases in temperature [21]. The precise implications of this will be discussed in later sections of the paper. In contrast with the deep anoxic occurrence of hydroxylated archaeol, the Thaumarchaeota producing hydroxylated GDGT seemingly stick closer to the surface of the ocean, which is why the ring index of these lipids has been proposed as a robust way to trace historical sea surface temperatures [21, 22]. This shows hydroxylation is not bound to either oxic or anoxic metabolisms.

Hydroxylation in archaeal lipids is hypothesized to affect membrane fluidity by increasing the polar surface area of the membrane and creating hydrophilic pockets, effectively decreasing the size of the hydrophobic core [18, 23]. An interesting MD study investigated the properties of hydroxylated GDGT and found results in accordance with this hypothesis. They found the OH groups extend the polar head group region which introduces a local disorder that propagates along the lipid tail and introduces cavities deeper in the hydrophobic core of the membrane. These effects resulted in increased permeability of solutes at low temperatures [17]. If we consider hydroxylation as a modification that causes a more disordered membrane and increases permeability, we can see why this modification is useful in colder environments where low temperatures can make membranes more rigid and impermeable than might be desired. Interesting questions remain on how this modification compares to unsaturated lipids, which introduce disorder in a similar manner and are used by bacteria

under similar conditions, and how choices between these two modifications are balanced with the need for an impermeable membrane able to prevent uncontrolled leakage.

4 Saturation

Although unsaturated lipid tails are very common in bacterial membranes, they are noticeably absent in most archaeal membranes. Part of this is likely due to the fact that isoprenoid tails have a much more gradual phase transition between the ordered and disordered phase that does not have to be regulated using unsaturated lipids in the same way as in bacterial membranes [24]. Nevertheless, unsaturated lipids do occur in archaeal membranes, most commonly in Halobacteria. Unsaturated lipids outside of Halobacteria are seemingly much rarer, although a few methanogens containing unsaturated lipids have been found [15, 25].

Unsaturated GDGTs have so far not been found. This and the rarity of unsaturated archaeol are somewhat surprising as unsaturated double bonds naturally occur in precursors for both archaeol and GDGT, although during synthesis of GDGT some of these bonds are likely sacrificed in linking the two diethers together, meaning no double bonds could be present near the center of the lipid [26]. The other unsaturated double bonds are either used to synthesize the cyclopentane rings or are removed by Geranylgeranyl reductases [3, 11]. The absence of cyclopentane rings near the center of the lipid thereby further supports the hypothesis that no unsaturated bonds can be present near the center of GDGT as an effect of their synthesis pathway. The use of reductases also means that unlike in bacteria, where unsaturated bonds are actively introduced, unsaturation is a passive event where lipids are incompletely saturated [18]. Therefore, unsaturation in GDGT and archaeol is actively avoided by most Archaea.

Unsaturation in archaeal lipids generally seems to be used as an adaptation against salinity or cold. As a response to cold, a short term dynamic increase of unsaturated archaeol has been observed in species of both Halobacteria and methanogens when grown at lower temperatures [15, 27]. The function behind this is most likely in line with the way unsaturation is used in bacteria: low temperatures cause membranes to become too rigid, which is counteracted with the introduction of unsaturated bonds that cause disordered kinks in the structure of the lipid. As previously mentioned, in isoprenoid chains this is usually much less of a problem, which is most likely why it is only used in Archaea under more extreme psychrophilic conditions.

Although it might at first seem unintuitive that more disorder is introduced in a saline conditions where permeability is preferably kept low, both a short term dynamic increase in unsaturation and a more long term species specific trend between optimal salinity and unsaturation has been observed [28, 29]. As a mechanism, unsaturated lipids are speculated to deal with the dehydration of the head groups in highly saline environments, which decreases membrane

fluidity and can potentially cause membrane disrupting phase transitions [30]. Outside of its use in Halobacteria, this function of preventing dehydration might be at play in *Methanopyrus kandleri*, a unique hyperthermophilic methanogen with a membrane composed exclusively of archaeol, with roughly 8% of the lipids fully unsaturated. Its use of unsaturated lipids does not make sense when only temperature is considered, but *Methanopyrus kandleri* also grows under hypersaline conditions. It might therefore use unsaturation to prevent these phase transition effects, although its unsaturation is usually explained as a primitive feature coming from its deep short lineage in the phylogenetic tree [31].

As previously mentioned, unsaturation generally works through the introduction of disorder, although with regard to permeability unsaturation can follow a more complex pattern, where introduction of a single unsaturation slightly decreases permeability while a more fully unsaturated chain increases permeability [28, 32]. Regardless of this effect, unsaturation seems to be used generally whenever a more disordered membrane is needed, be it against rigidifying effects of salinity or low temperatures. This contrasts with the much more specific use of hydroxylation which seemingly mostly occurs as a cold response. Despite this, hydroxylation seems more widespread among different branches of Archaea than unsaturation, even though unsaturation would be easier for Archaea to produce since they are a passive byproduct of lipid biosynthesis. One Archaea living in extremely saline and cold conditions has been reported to exhibit both unsaturation and hydroxylation, suggesting they serve complementary yet slightly different functions [33].

5 Extended tails

In some species of Archaea, archaeol can have extended C25 tails as compared to the usual C20 tails. Extended tails are characteristic of Halobacteria and with a few exceptions are almost entirely exclusive to this group. In Halobacteria, archaeol featuring this modification have a single extended C25 tail accompanied with a normal C20 tail, with double extended tails only present in tiny amounts [12]. The use of extended archaeol seems more strongly linked to the phylogenetic history and the unique survival strategy of Halobacteria rather than it being a more widespread adaptation. As the exception to this story, the hyperthermophilic Cenarchaeote *Aeropyrum pernix* has membranes fully composed of double extended tails [34].

The unique use of double extended tails in *Aeropyrum pernix* seems to be an adaptation to the high temperature and low pH in which the organism is found and seems relatively unrelated to the way extended tails are used in Halobacteria [35]. The double extended tails make up the core lipid and give this organism a very thick membrane that only becomes liquid crystalline at temperatures above 40 degrees Celsius, resulting in a fluidity and permeability similar to the GDGT monolayers found in other hyperthermophilic Crenarchaeota [36]. It is currently unknown how exactly this double extended lipid bilayer differs in

properties from GDGT and why *Aeropyrum pernix* uses this adaptation over the GDGT monolayer more common among its fellow Crenarchaeota.

The use and mechanism behind the single extended tail found in Halobacteria seems to be somewhat different. Instead of being an adaptation against high temperatures, Halobacteria containing higher concentrations of extended archaeol on average grow under higher optimal salinity and pH [28, 37]. As opposed to double extended tails, which simply use the increased membrane thickness as a way to decrease permeability and fluidity, The use of extended tails in Halobacteria has been proposed to work through the C25 and C20 tails interlocking into the C20 and C25 tails of the opposite leaflet, creating a sort of ‘zipper’ effect [29, 38]. This ‘zipped’ core has an increased amount of van der Waals interactions between lipids, strongly decreasing permeability. The reason these extended tails are used to decrease permeability as opposed to the more common GDGT likely has to do with the phylogenetic history of Halobacteria [13].

One hypothesis regarding the history of Halobacteria involves the accommodation of their membranes to their uniquely aerobic metabolism when compared to the anaerobic methanogens to which they are closely related. It was found that the genes for this aerobic metabolism were likely acquired through horizontal gene transfer from bacteria which evolved their metabolism after the oxygen revolution [39]. Because of this they have a complex electron transport chain similar to those in mitochondria and chloroplasts [29]. Kellerman et al. Hypothesize that it were these genes that caused Halobacteria to give up GDGT for the significantly more fluid extended tails, considering that electron transport through the membrane is the rate limiting step in both chloroplasts and mitochondria [40, 41].

This tension between hypersaline conditions requiring a highly impermeable membrane, and the newly acquired respiratory genes requiring a highly fluid membrane are then likely what led Halobacteria to evolve their unique use of extended tails. They use the zipper effect of extended tails to provide a relatively fluid core that nevertheless has an impermeability close to GDGT [28, 30]. Unsaturated bonds can then be used to further optimize membrane fluidity. The question remains how these two adaptations interact. It might be the case that Halobacteria rely on the previously mentioned non-linear effect where small amounts of unsaturation can actually decrease permeability. Another option might be that if impermeability in Halobacteria is mainly maintained using the zipper effect of extended tails, saturated bonds away from this zipper region and closer to the surface can introduce a high degree of fluidity for components of the respiratory system without significantly affecting permeability. This would also explain why unsaturation is common in Halobacteria, but is very rare outside of it.

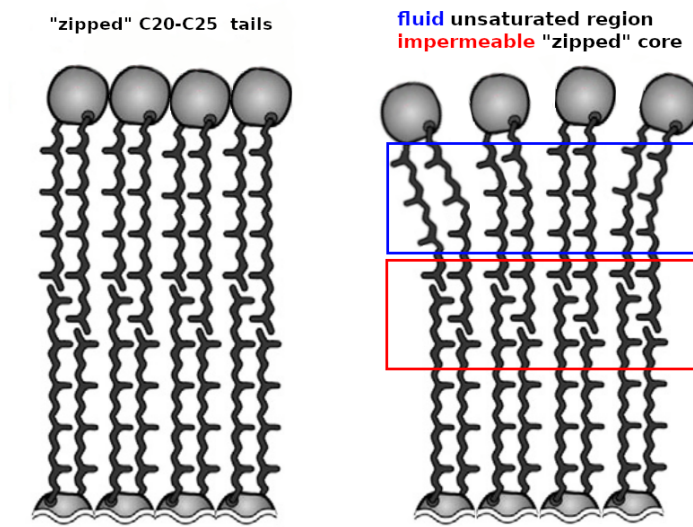


Figure 3: the zipper effect of extended tails and their possible synergy with unsaturated bonds. figure freely adapted from Kellerman et al.[29]

6 Macrocyclic structures

Macrocyclic structures consist of a crosslinking of the two tails in the middle of the membrane. For macrocyclic archaeol (MAR), this link is located at the end of the tails. When GDGT has such a link located in the middle of the lipid it is often called GMGT (or H-GDGT due to the crosslink giving the lipid a H-shaped structure). Macrocyclic archaeol and GMGT are both widespread and not particular to any specific phylogenetic branch [42]. Very high concentrations of macrocyclic archaeol might be considered characteristic of Methanococcales, although many methanogens and a few Archaea outside of the methanogens also produce macrocyclic archaeol [42].

Increase of macrocyclic archaeol as a short term response to an increase in temperature has been reported in multiple species of Archaea [9, 43]. As a long term trend more generally, many of the species featuring this modification grow under high optimal temperatures [42, 44]. Macrocyclic archaeol has experimentally been shown to have significantly lower permeability compared to normal archaeol, and the covalent bond at the end of macrocyclic archaeol also drastically decreases motion in the membrane [32]. This helps compensate for the large increase in fluidity and permeability that comes with higher temperatures. Since these decreases in fluidity and permeability are generally similar albeit somewhat milder than the properties of GDGT, the question is raised what precise properties macrocyclic archaeol has that are not simply a milder version of GDGT [28, 45]. One of these unique properties might be its relation to pressure.

Macrocylic archaeol has since very early on been speculated to be a response to pressure due to its frequent occurrence in Archaea living in deep sea thermal vents [44]. Its use as a short term response against pressure occurs in several species of Archaea [9]. Some methanogens show an interesting behavior where they decrease GDGT concentration as a reaction to an increase in pressure, and instead greatly increase the concentration of macrocylic archaeol [46]. This suggests that macrocylic archaeol is not simply a milder reaction to the same external conditions as GDGT, but indeed has unique properties in its own right.

This strong response to pressure might indeed be due to a unique structural feature of macrocylic archaeol, although not much actual research is currently available on its precise properties. Dannenmuller et al. found liposomes composed of macrocylic archaeol showed properties usually associated with negative curvature lipids, suggesting its bulky crosslinked core might induce a relatively large negative curvature [32]. Considering that high pressure has been found to increase the positive spontaneous curvature of membranes, it might be the case that some Archaea use the negative curvature of macrocylic archaeol to compensate for this effect under high pressures [47].

Compared to macrocylic archaeol less is known about the exact properties of GMGT, although its relation to high temperatures is well established. GMGT generally occurs in two different environments: The first is in a hyperthermophilic setting near geothermal vents, where GMGT is likely used as response to high temperatures, although no clear correlation between differences in temperature and GMGT in these hyperthermophilic settings was found [42, 48]. The second is in more mesophilic marine environments and peat bogs, where despite the overall lower temperature, a stronger positive correlation between temperature and GMGT was present [49]. GMGT is related to temperature in both cases, although its relation in both appears to be slightly different. In the first, GMGT is used as a general adaptation to hyperthermophilic conditions, in the second, as a more fine tuned response to minor differences in temperature.

7 Cyclopentane and cyclohexane rings

Within the membrane spanning structure of GDGT tetraethers, one common modification is the addition of cyclopentane or cyclohexane rings. In the case of cyclopentane, 1-8 rings can be added throughout the tetraether except near the middle, and are usually called GDGT-0 to GDGT-8. The presence of cyclopentane is widespread wherever GDGT is also present. It is present in all Thaumarchaeota, and is common in many Crenarchaeota and metalogens [11]. Besides cyclopentane, a cyclohexane ring can also be present near the middle of the tetraether, in which case it is referred to as crenarchaeol. Despite the name, crenarchaeol does not occur in Crenarchaeota and is instead unique to Thaumarchaeota [50].

The use of cyclopentane rings as an adaptation to extreme conditions is widely attested and for a long time was thought to be its only use. As a short term response, many species in thermophilic or hyperthermophilic conditions are

known to raise their ring count as a response to a raise in temperature or pH [9, 44]. Especially higher ring counts almost exclusively occur in hyperthermophilic conditions [6]. Yet the presence of these rings is not only used for dealing with extreme conditions. The fact that all Thaumarchaeota have these rings, and a subbranch of Thaumarchaeota even contain an additional cyclohexane ring, suggests that despite its use in extreme conditions, the rings can also serve another function.

The chemically stable tetraethers of GDGT and its varying ring counts are often preserved in ocean sediments. In these sediments, mostly generated by Thaumarchaeota, there is a strong correlation between ring count and ocean temperature, allowing researchers to glean historical information about the environment [51]. The most popular of these indexes is called TEX86, although others like the previously mentioned ring index of hydroxylated GDGT are also in use. Important to note is that the temperature fluctuations to which the Thaumarchaeota adapt their ring count are very subtle, only a few degrees in the overall ocean temperature. This tells us that much like the use of GMGT, its uses can be split into a extremophilic survival mechanism on the one hand, and a fine-tuned optimization to mesophilic conditions on the other.

Regarding the precise mechanism, the rings are believed to allow for closer packing, thereby decreasing permeability and stabilizing the membrane [18, 52]. This is confirmed by multiple MD papers which studied the effect of introducing cyclopentane rings into the membrane [53, 54]. In experiments with liposomes however, the precise number of cyclopentane rings was found to not be correlated with permeability, although this has been speculated to be a side effect of the way liposomes are tethered in the experimental setup [55, 56]. One paper speculates on a nonlinear effect of cyclopentane rings on the packing of the membrane, with the tightest packing near optimal growth temperatures, which might explain why its precise effect on permeability is hard to pinpoint [57]. cyclohexane has much more prominent effect and showed a 40 percent decrease in permeability, likely due to the flexibility of the larger ring allowing for tighter packing [55].

Another use of rings might be their role in the energetics and metabolism of the cell. By decreasing the permeability of the membrane, you also decrease the energy cost of cellular maintenance. On top of that, cyclopentane rings are likely synthesized using the unwanted double bonds present during the lipid synthesis of archaeal lipids [58]. This reaction does not require an electron donor, while removing the double bonds through Geranylgeranyl reductases does. In conditions with low availability of nutrients and electron donors like oxygen, the addition of rings will therefore be favorable. It has indeed been experimentally demonstrated that reducing the availability of oxygen increases the TEX86 index by roughly 10 degrees in some cultures of mesophilic Archaea [59].

This use of cyclopentane synthesis as a way of saving electron donors might also be why it is more widespread than cyclohexane despite cyclohexane showing a greater decrease in permeability. While the mechanism for the incorporation of cyclohexane is not yet known, cyclohexane occupies a position near the mid-

dle of GDGT, meaning the unsaturated bonds would already be used in creating the tetraether structure itself [11]. It therefore probably does not rely on these saturated bonds, and requires a more active energy input from the organism than cyclopentane rings. It is seemingly only with Thaumarchaeota where incorporating cyclohexane become viable despite its higher costs. Keeping in mind these facts regarding the fine-tuned use of cyclopentane and -hexane in mesophilic conditions and its possible relation to nutrient poor conditions will be useful when investigating the precise role of GDGT and its relation to Thaumarchaeota.

8 GDGTs

GDGTs are incredibly widespread among Archaea to the point that it is sometimes listed as one of the defining features of archaeal membranes alongside the previously mentioned ether bond, isoprenoid chains and reversed stereochemistry [4]. They are therefore not particular to any one branch of Archaea. In practice, the majority of GDGT in oceans tends to come from Thaumarchaeota, while in lakes they usually come from a mixture of Thaumarchaeota and methanogens [58, 60]. In some ranches of Archaea GDGT is actively avoided, like in Halobacteria, but these form the exception rather than the rule.

For a while GDGT was thought to be exclusive to extreme conditions, and many of its properties support this hypothesis [5]. Liposomes containing tetraethers are found to be more stable at high temperatures and their high impermeability helps to maintain homeostasis at extreme conditions like high temperature or low pH [59, 61, 62]. Further support comes from a comparison of optimal growth temperatures and acidity, which are significantly hotter and more acidic for Archaea with membranes fully composed of GDGT when compared with those fully composed of archaeol [9]. Short term responses like upregulation of GDGT as a response to rises in temperature have similarly been reported in multiple species [43, 63, 64]. With its use these extreme conditions well established, the question of how and why they are used in the mesophilic Thaumarchaeota does remain.

One hypothesis is that it is simply an evolutionary leftover from its thermophilic past. This is supported by evidence suggesting Thaumarchaeota had a thermophilic ancestor that only later adapted to a mesophilic environment through horizontal gene transfer [65]. However, recalling that the number of cyclopentane or hexane rings in Thaumarchaeota are very deliberately fine tuned to minor fluctuations in temperature, and that these rings are used to even further increase membrane impermeability when needed, it seems unlikely that the presence of GDGT in Thaumarchaeota is simply a now-redundant leftover from their thermophilic past. Rather, the very rigid and impermeable structure of GDGT and its cyclopentane rings might have come to serve a different function for Thaumarchaeota after their switch to a mesophilic environment.

The most likely of these new functions is that the impermeability and rigidity coming from GDGT are used to prevent wasteful ion cycling [66]. The

presence of rings in Thaumarchaeota and their role in further reducing permeability support this hypothesis [59]. Thaumarchaeota have an ammonia-oxidizing metabolism, and are therefore reliant on the presence of both oxygen and ammonia, both of which can be very scarce in the oceanic waters in which Thaumarchaeota thrive. The choice to maintain a highly rigid, impermeable membrane in mesophilic conditions might therefore be an adaptation to an extremely low availability of energy sources, so that the speed of metabolism and reproduction is sacrificed in order to maximize efficiency.

The fact that the rigidity of GDGT can be a significant sacrifice to other metabolic and reproductive functions is reflected in the earlier mentioned hypothesis that Halobacteria gave up GDGT to more effectively use new metabolic pathways even though these Archaea grow in extremely halophilic environments. In conditions of low nutrient availability, the downsides of a slower metabolism and reproduction are less significant, since these organisms would often not have the resources to reproduce at a faster rate to begin with. Seen like this, Thaumarchaeota are in a way still extremophiles, but the extreme conditions in this case are not salinity or temperature, but scarcity of nutrients.

9 Glycosylated headgroups

: Besides these modifications to their core lipids, Archaea also employ different polar headgroups as an adaptation to their extreme environments. Similar to Bacteria and Eukaryotes, the two main types of polar headgroups in Archaea are either a phosphatidyl or a sugar moiety. Archaea to a great extent feature the same phosphatidyl groups as bacterial membranes, consisting of phosphocholine (PC), phosphoethanolamine (PE), phosphoglycerine (PG) and phosphatic acid (PA). The balance of which is depends on temperature in order to keep a stable surface tension [67]. When it comes to GDGTs, these different phosphatidyl moieties do not seem to have a significant effect on permeability compared to the impermeability already caused by the rigid tetraether core itself [55]. The sugar moieties on the other hand have a more significant effect on permeability. Although glycosylated lipids are common in almost all branches of Archaea, they are particularly abundant in methanogens and thermoacidophiles [68]. In methanogens, sugar headgroups occur exclusively on GDGT, and are present asymmetrically, with the phosphatidyl moiety on the cytosolic side and the sugar group directed to the outside [68, 69].

These sugar headgroups are often employed in thermophilic and acidophilic conditions because they help to stabilize the membrane against osmotic and temperature stresses through a network of hydrogen bonds in the glycosyl head groups [70]. An increase in these sugar groups has indeed been reported as a short term response to high temperatures and low Ph in thermoacidophilic euryarchaeota, and experiments found liposomes containing two or more sugar groups had a significantly lower proton permeability than those with one or less [71]. The downside of introducing these sugars is that this network of hydrogen bonds results in a strong decrease in lateral diffusion, indicating there is likely

a payoff between permeability and rate of diffusion [72]. This is confirmed by research showing that methanogens and other thermoacidophiles produce a significantly higher amounts of glycolipids in nutrient poor conditions, where the payoff shifts more towards impermeability for the sake of efficiency [73, 74]. the asymmetric polar headgroups used by methanogens might therefore also be a way of minimizing the downsides of the low fluidity by only employing it on one side of the membrane, although this remains to be further investigated.

Within ocean waters and sediments, many glycosylated lipids from methanogens and thaumarchaeota are found, showing that they are also used in more mesophilic conditions, likely also for reasons of low nutrient availability. One lipid of note here is HPH-crenarchaeol, a crenarchaeol with hexose sugars on both sides of the lipid core. This lipid was found to be unique to ammonia oxidizing organisms, and strongly covaries with thaumarchaeotal gene abundances, indicating that these are mainly produced by thaumarchaeota. [50] Introducing sugars on both side of the membrane is likely to reduce the permeability even further at the cost of membrane fluidity, something in line with our hypothesized strategy for thaumarchaeota of prioritizing impermeability over speed of metabolism due to nutrient poor conditions.

10 Anionic headgroups

Besides glycosylated lipids, Halobacteria and other halophiles also use other adaptations unique to their extreme conditions. As we saw in the sections on unsaturation and extended tails, Halobacteria require both an impermeable membrane to survive the extreme saline conditions, and a membrane fluid enough to utilize the electron transport chain they obtained through gene transfer. The tension between these two requirements is also reflected in the specific use of anionic headgroups found in these archaea.

Halobacteria feature high amounts of anionic headgroups, with the cardiolipids and lipids with a Me-PGP headgroup even containing two negative charges, likely as an adaptation to high cation concentrations [75]. With the exception of alkalophilic Halobacteria, these often also feature sulfonate-containing headgroups which are also negatively charged [13, 69]. Due to the preferential attraction of K ions to sulfate, it has been speculated that Halobacteria use these sulfated lipids to attract K ions near potassium pumps in order to better maintain osmotic pressure necessary to live in hypersaline environments [76]. The negative charges of sulfates and other anionic headgroups are also speculated to affect membrane fluidity by preventing the lipids from clustering closely together due to the repulsion of the negative charges [9]. Large headgroups like Me-PGP and the sulfonate headgroups similarly help by preventing the lipids from packing too closely through their bulk [75, 77].

Although these aspects might play into the use of anionic headgroups, another perhaps more important aspect comes from its interaction with divalent cations like magnesium. It has been found that fluctuations in the amount of divalent cations significantly influence the headgroup composition of *Haloferax*

volcanii while for fluctuations in monovalent cations it remains mostly the same [29]. In the case of lipids with a single negative charge, divalent cations like magnesium lodge between the charges and form a highly rigid network of electrostatic interactions, similar to the network of hydrogen bonds present in glycosylated lipids [29]. However, when magnesium concentrations become too high this network can quickly become too rigid. The two charges of Me-PGP are able to compensate for this by binding a single divalent cation without forming a network with other lipids. This way, the amount of Me-PGP is used in order to keep the membrane fluid at high magnesium concentrations. At medium or low levels of magnesium, the Me-PGP is much lower, and eventually replaced by cardiolipins, which help maintain impermeability in the absence of magnesium [29].

11 Conclusions

When looking at all different modifications using physio-chemical research coming from MD and liposome experiments, as well as research regarding both the long term survival strategies and short term adaptations in the Archaea exhibiting these adaptations, it allows us to start piecing together the different ways in which each modification is used by different species of archaea and why. In the introduction it was noted that especially the use of GDGT and its cyclopentane rings was pulled into question after the discovery of mesophilic Thaumarchaeota. But when comparing and contrasting the different uses of lipid modifications and their use in various survival strategies adopted by Archaea, we can now speculate about certain uses of modifications that could not have been made otherwise.

The first half of the paper we mainly examined modifications related to high fluidity. Looking at hydroxylation, Archaea seemingly use it mainly as a response to cold. Comparing this with the use of unsaturated bonds allowed us to note the relative rarity of unsaturation in Archaea compared to hydroxylation despite their fairly similar mechanism. The rarity of unsaturation is especially striking since they are a natural byproduct of archaeal lipid synthesis, and require additional, sometimes costly steps to remove. Unsaturation does frequently occur in the membranes of Halobacteria, which due to their unique evolutionary history evolved highly fluid yet impermeable membranes for which they rely on the unique zipper effect of extended tails. We speculate that these tails maintain their impermeability mainly through the zipper effect of extended tails in such a way that unsaturated bonds outside of this "zipped" core can increase fluidity without significantly affecting impermeability.

In the second half, we mainly examined modifications related to high impermeability. Macrocyclic archaeol forms one of the possible responses to extreme heat and acidity available to Archaea. Comparing it to GDGT raises the question what precise niche macrocyclic archaeol fills that is not already fulfilled by GDGT. We speculate that one of its unique uses might be its use as a response to pressure due to its possible negative curvature. After looking at the proper-

ties of GDGT and its ring moieties, and how these relate to other modifications, we could better speculate about its use in mesophilic settings. We found a twofold use, either as a survival mechanism against extreme temperature and acidity, or as a way to preserve energy in mesophilic conditions. It is this last use that allows us finally to answer why the mesophilic Thaumarchaeota feature membranes so rich in GDGT and high ring counts. It is likely the case that Thaumarchaeota live under extremely nutrient poor conditions, forcing them to minimize use of electron donors in removing the unwanted saturated bonds and minimize maintenance costs that come with a permeable membrane. Lastly we looked at different uses of headgroups in Archaea, where sugar headgroups are used to introduce a rigidifying effect that helps maintain an impermeable membrane in thermoacidophilic conditions and conditions of nutrient scarcity, and anionic headgroups in Halobacteria seem to be used to retain a fluid yet impermeable membrane in varying concentrations of divalent cations.

12 References

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