Deciphering the Tetraether lipid biosynthesis pathway in Archaea
(Looking for an enzymatic alternative to the metathesis reaction)

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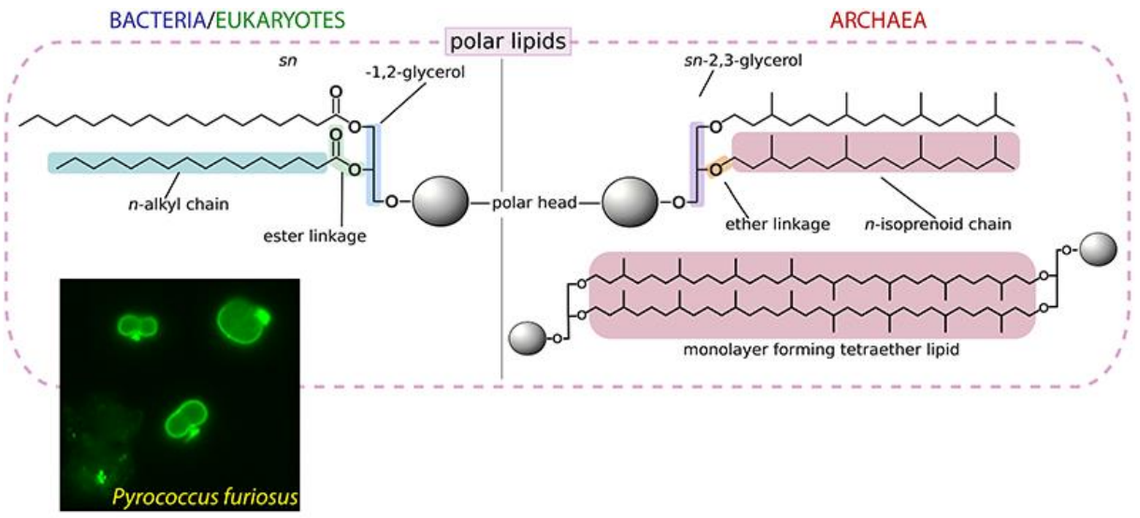
The three domains of life are split in two by the great lipid divide, separating bacteria and eukarya from the Archaea. The former produce the classical glycero(phospho)lipids, in which acyl chains are ester bound to a glycerol backbone. The lipids of the later are composed of polyisoprenoid hydrophobic chains ether linked to the glycerol backbone. Archaea were originally discovered in extreme environments, and are often considered as extremophiles. Indeed, their specific lipids are central to their adaptation to these difficult conditions. These form membranes, exhibiting more rigidity, lower permeability to water and protons and increase thermal resistance. In addition, Archaea produce bipolar, tetraether lipids, for a further increase in these parameters. Tetraether lipids allow Archaea to always be the last organisms in the most extreme biotopes. If the synthesis pathway of archaeal monopolar lipids is now well characterized, the tetraether synthesis pathway remains one of the last enigma in archaeal biology. Two hypothetical tetraether lipid biosynthesis pathways have been proposed, based on radioactivity incorporation or on comparative genomics. Regardless of their validity, both pathways require the condensation of the terminal olefin groups of the isoprenoid chains.



Hence, we hypothesize that the condensation of these two olefins would require a free radical mechanism, which is likely to be carried out by a radical S-adenosylmethionine protein (HHC). To search for this activity, we propose to synthesize labelled tetraether lipid precursor analogs to allow the development of a functional screen. These analogs will vary along two lines: first, they will harbor isoprenoid chains of variable lengths, from 1 to 4 units, second, the isoprenoid chains will or will not be linked to a glycerol backbone. Hence, the precursors will vary from a single isopren unit directly linked to a fluorochrome to a molecule harboring two phytanyl chains, analog to the archaeal diether lipid. Thus, our precursor analogs will include all putative precursors of the two putative tetraether biosynthesis pathways. Using this screen, we will search for the HHC activity in three different libraries of the hyperthermophilic archaeon *Pyrococcus furiosus*: a collection of putative SAM mutants, a random mutant library, and a *P. furiosus* expressed-gene library; identify the genes coding the HHC activity, and fully characterized them. As an alternative approach in case the HHC assay failed, we will use immobilized

precursor analogs to purify *P. furiosus* proteins with affinity to polyisoprenoids or archaeol, identify the coding genes and characterize the proteins.

In a second step, HHC will be used to produce tetraether lipids in order to study the contribution of diether and tetraether lipids in archaeal membranes, measure the membrane parameters in membranes of known diether and tetraether lipid compositions, with a special focus on the spatial distribution of the di/tetraether lipids within mixed membranes. This data will complete the molecular ultrastructural model of the archaeal membrane that we have developed and allow to interpret membrane evolution of high temperature and low pH adaptation in the phylogeny of Archaea.

The main expectation from this project is the deciphering of the archaeal tetraether lipid biosynthetic pathway, which is of major fundamental interest.

Last, the condensation of the olefin groups resembles the metathesis reaction, which has revolutionized organic chemistry. The identification of the HHC enzyme, and related proteins in the future, could bring catalysis in organic chemistry into the green age affording the opportunity to perform carbon-carbon in greener bio-catalyzed reactions. Hence, the project also aims at obtaining a cheap hybrid, economically relevant, synthesis scheme of tetraether lipids for the pharmaceutical industry.

Experimental work to be performed will include:

- Creation of specific and random mutants of *Pyrococcus furiosus*.
- Identifying proteins involved in ether lipid biosynthesis.
- Full characterization of said enzymes.
- Analysis of membrane lipid compositions by HPLC-MS
- Deciphering the physico-chemical behavior of reconstructed membranes (in collaboration)
- Stays in London, Dortmund, Grenoble, Bordeaux to measure membraen behavior.

Eligible applicants will have:

- A Master degree in microbiology, molecular biology or equivalent (delivered before September 2022
- **Practical experience of several experimental techniques in microbiology and molecular biosciences**
- **Good command of oral and written communication, in French and/or in English**
- **An interest in data analyses will represent additional assets.**

Work context and complementary information

The main objective of the M2E team is to understand microbial adaptative processes in prokaryotes thriving in extreme ecosystems, through a multidisciplinary approach ranging from physics to up-to-date environmental omics tools. The team focuses on Archaea, for which many novel phyla emerge daily and for which the ecology remains unclear, while our acknowledgement of their contribution to ecosystem functioning is increasing, and from which the team is developping novel, innovative biotechnological solutions.

Collaboration and workplace :

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