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Dianne Newman's Lecture Part 3: Interpreting Molecular Fossils of Oxygenic Photosynthesis

Teaching Tools were prepared by Jasper Williams.

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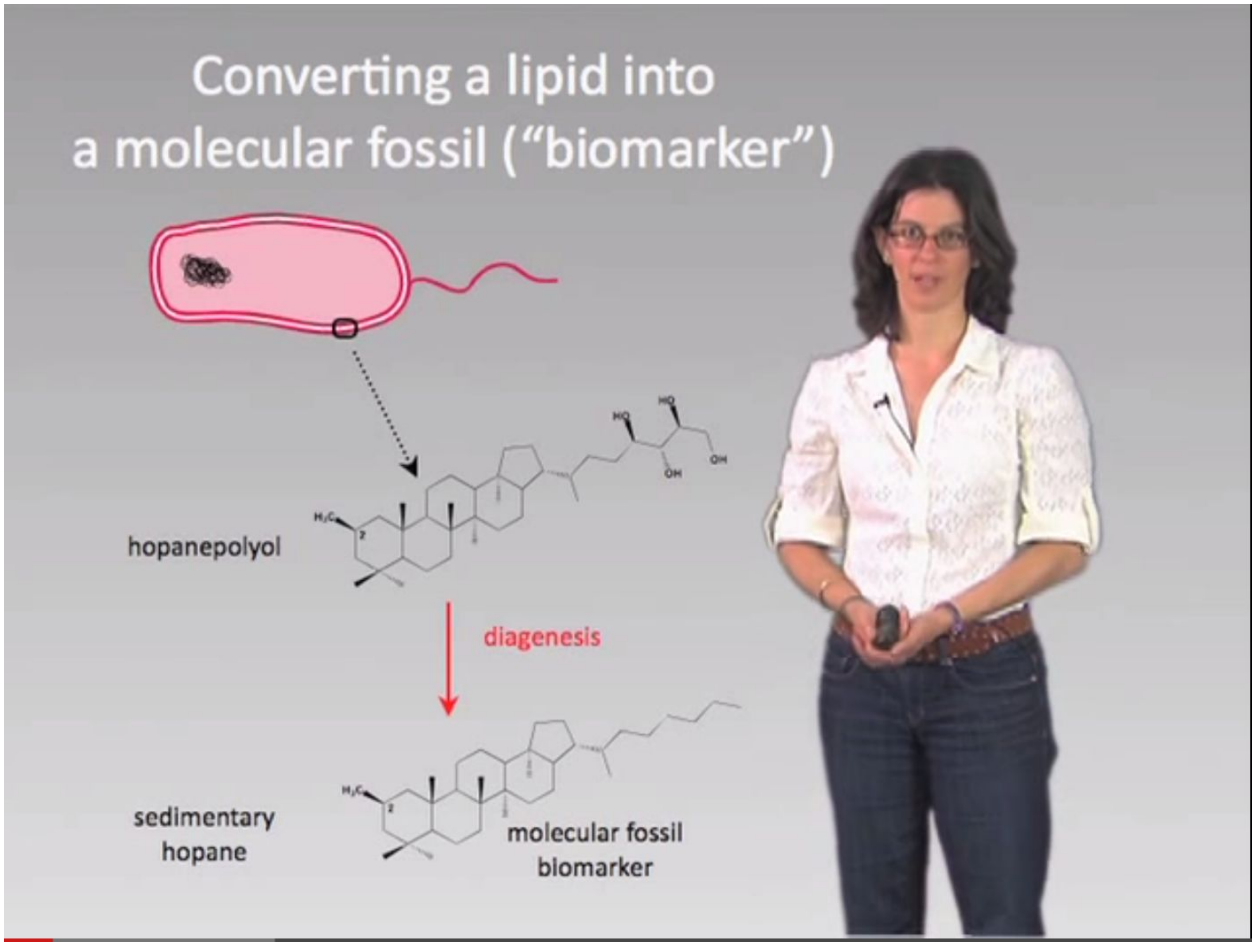
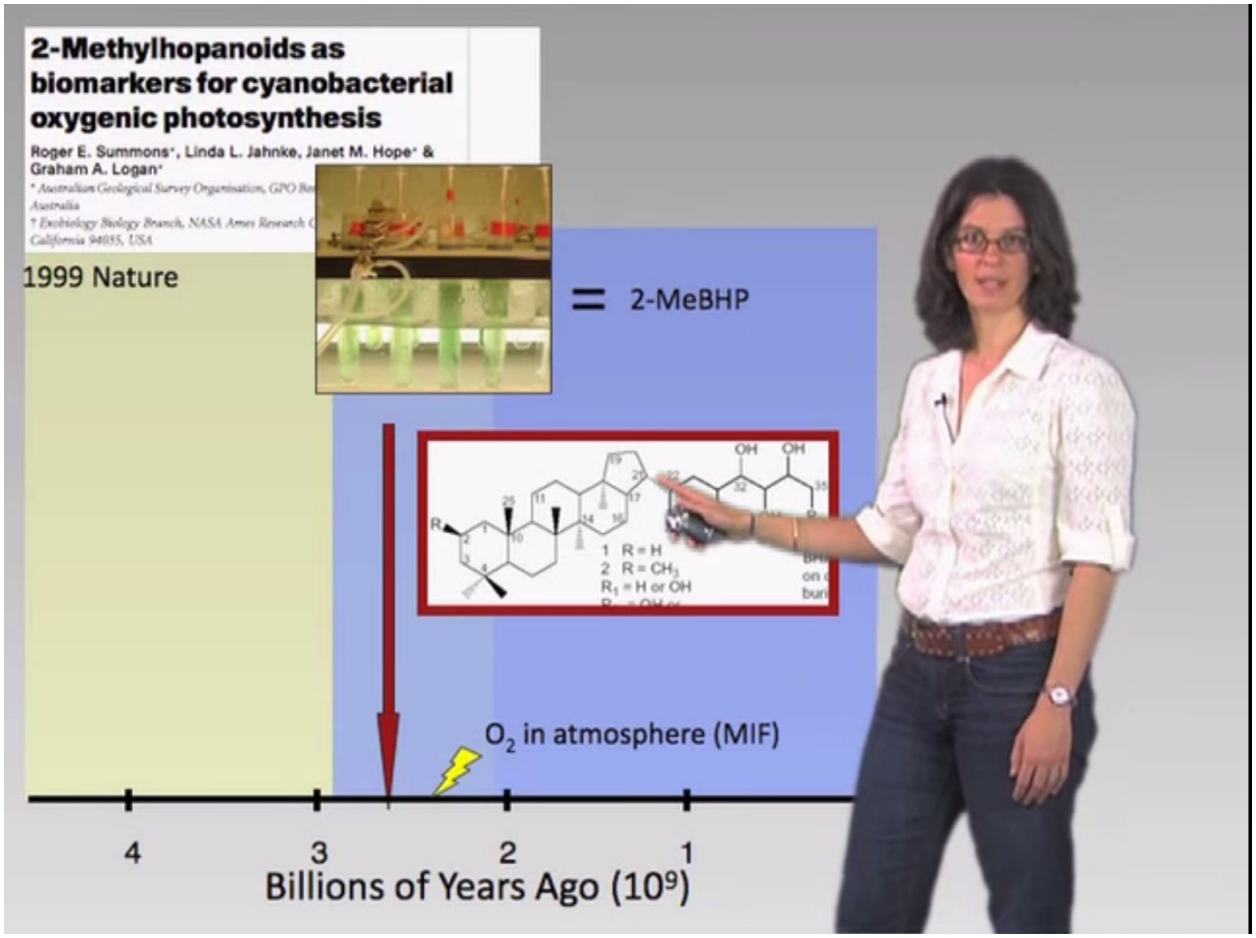
1. Keywords and Terms

photosynthesis, cyanobacteria, lipids, biomarker, evolution

2. Lecture Notes



Molecular fossils are organic molecules derived from once living organisms.
More generally, biomarkers are characteristics that indicate a modern or past biological process.
The Newman lab, and others, are particularly interested in investigating hopanes as potential biomarkers.
Hopanes are found abundantly in microorganisms and resemble sterols in eukaryotic cells.
Over time, sedimentation and diagenesis converts hopanepolyol into sedimentary hopane.



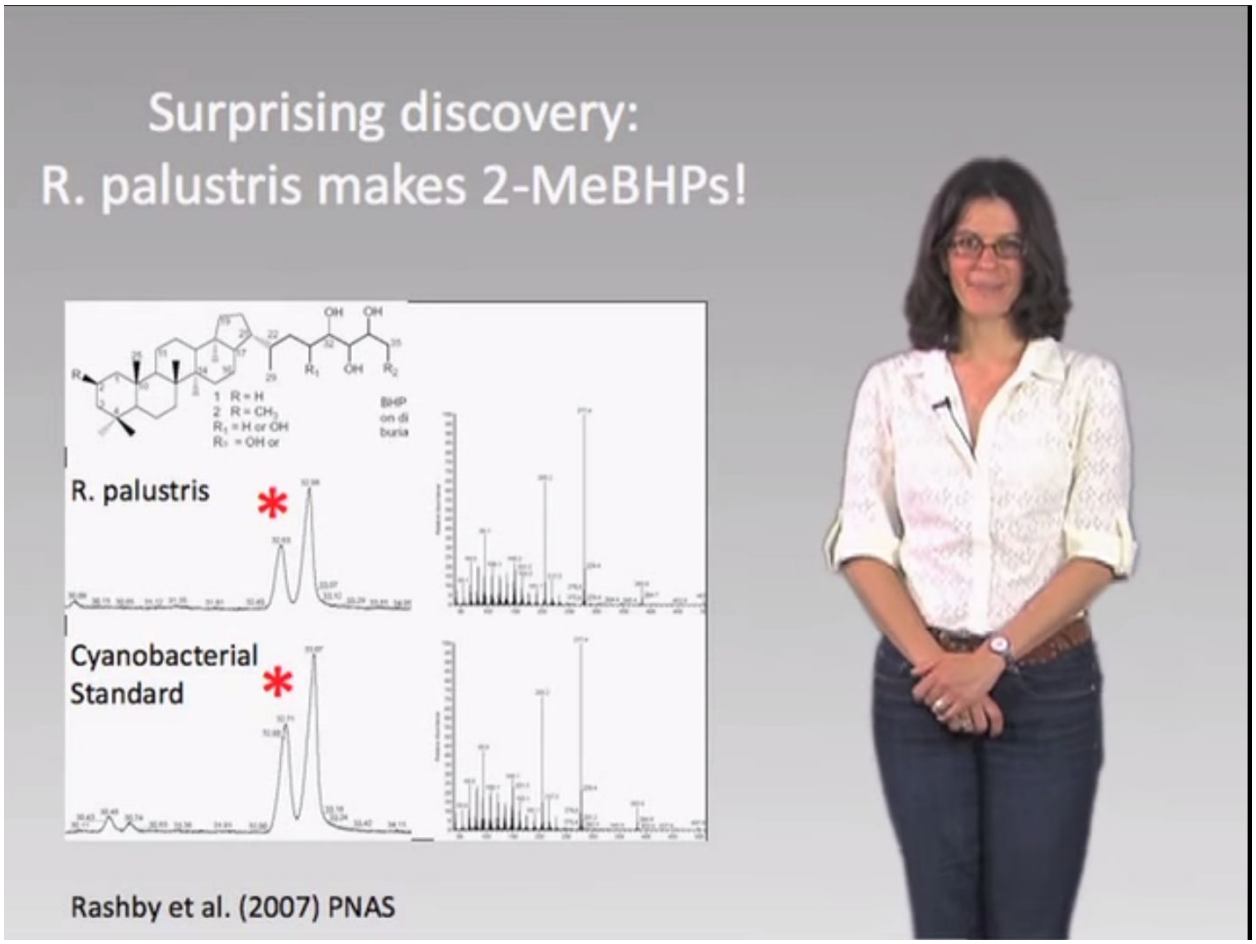
Oxygenic photosynthesis arose via a complicated evolu@onary scenario in which different classes of anoxygenic microorganisms combined via an as yet unknown mechanism.

Oxygenic photosynthesis is thought to have been “invented” by cyanobacteria.

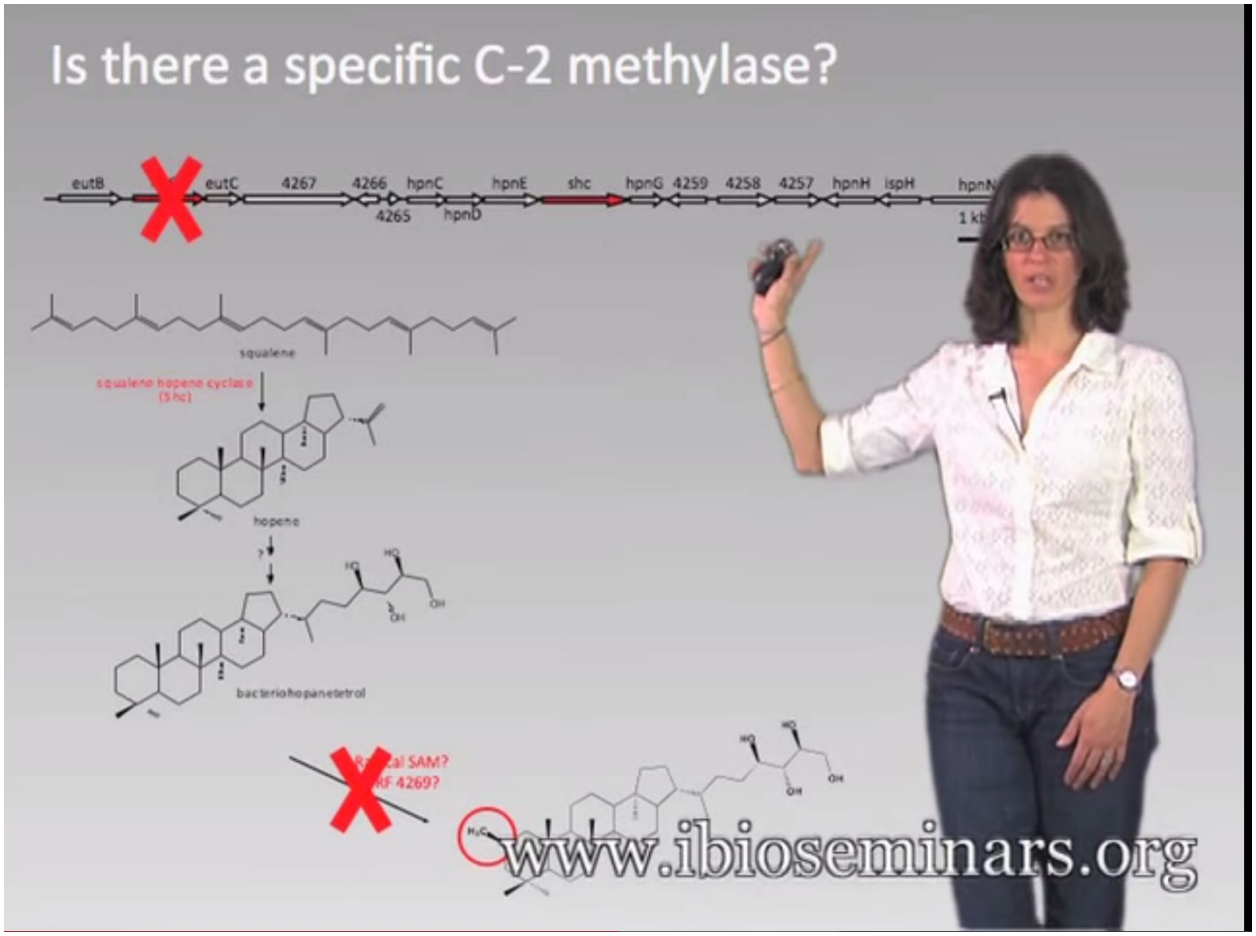
Geobiologists search in the rock record for markers that can be used as a signature for oxygenic photosynthesis.

Researchers looked at hopanoids as poten@al biomarkers for years, and for the past decade they have focused on whether 2-methylhopanoids can be used as biomarkers for oxygenic photosynthesis.

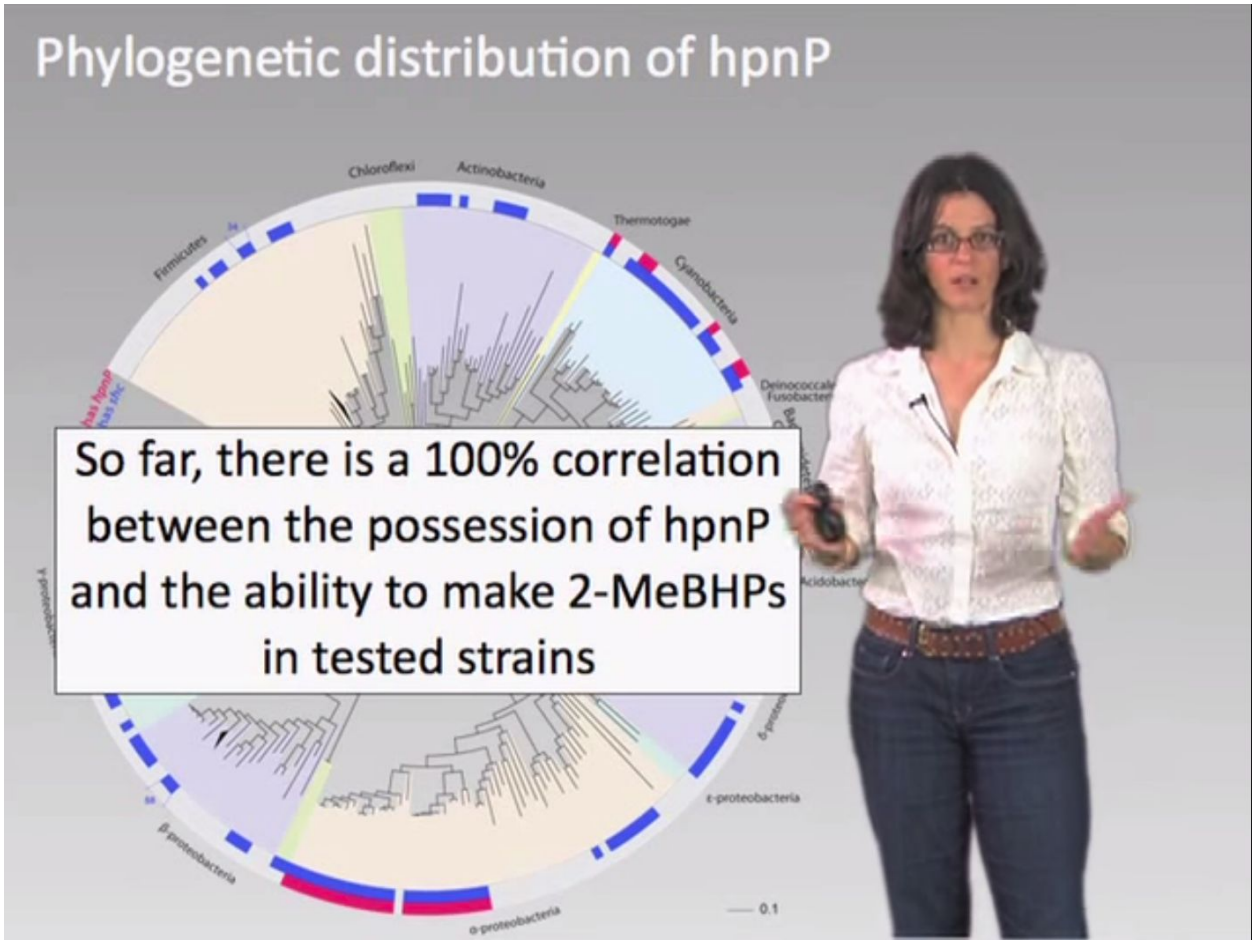
The focus on 2-methylated hopanoids followed a study in 1999 that concluded they could be used as biomarkers for cyanobacterial photosynthesis.



The Newman lab set out to scrutinize the conclusion that 2-methylated (2-Me) hopanoids could be used as biomarkers for cyanobacterial photosynthesis.
First, they asked whether 2-Me hopanoids have a unique distribution amongst modern organisms.
They collected environmental samples of diverse microorganisms and tested their ability to make 2-Me hopanoids.
One of their “negative control” strains *R. palustris* was able to make 2-Me hopanoids in concentrations as high as cyanobacteria, which indicates that 2-Me hopanoids cannot be used as a biomarker for cyanobacterial (and therefore oxygenic) photosynthesis.
They came to this finding only after testing 2-Me hopanoid production in a variety of growth conditions.



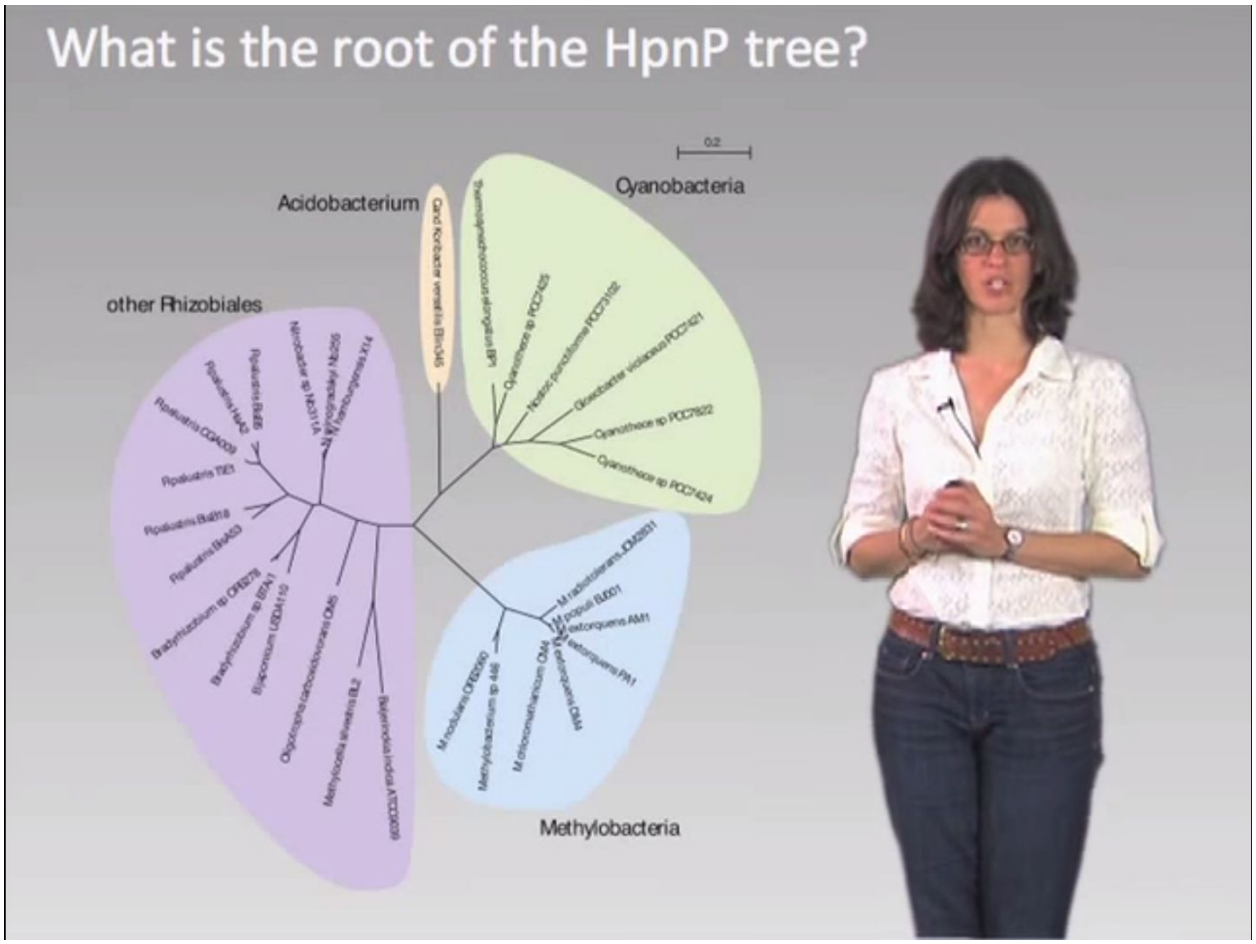
The finding that *R. palustris* can make 2-Me hopanoids opened the door for more detailed studies of the evolutionary history of 2-Me hopanoids.
R. palustris has an available genome sequence and can be easily genetically manipulated in the lab.
Using bacteriohopanepolyol (BHP) as a model hopane, the Newman lab asked whether there is a specific enzyme responsible for methylating BHPs at the 2 position.
After performing knockout and rescue studies, they determined that a single gene (hpnP) encodes the C-2 hopane methylase.



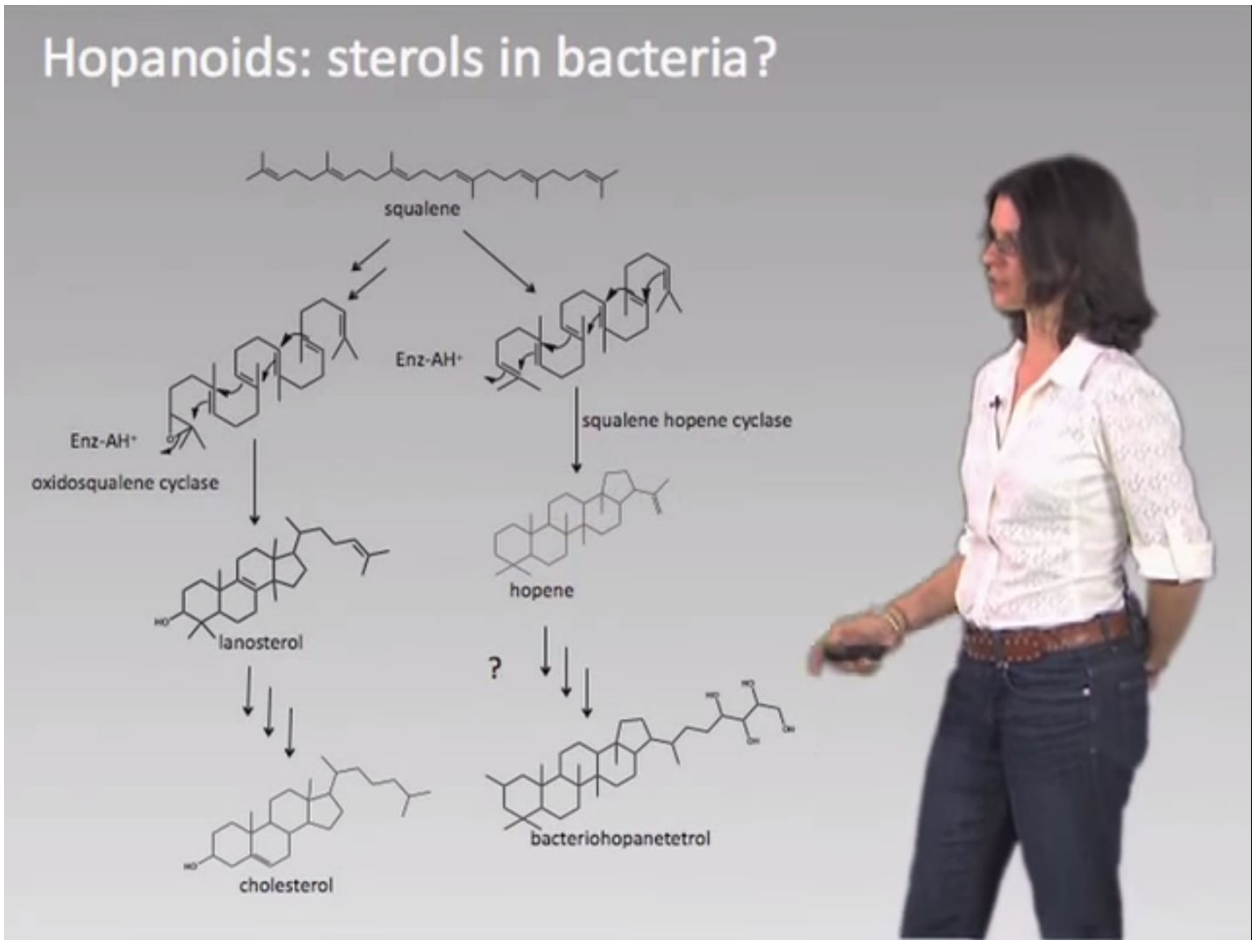
After discovering hpnP, the Newman lab explored its phylogenetic distribution and found that it was much more broadly phylogenetically-distributed than they initially suspected. In fact, hpnP was found to be present in many non-cyanobacterial organisms and not found in some cyanobacteria, indicating that this gene cannot be used as a marker for cyanobacteria.

However, they did find a 100% correlation between the possession of hpnP and the ability to make 2-Me hopanoids.

While comparisons were only made to already-sequenced microorganisms, the presence of hpnP appears to be a robust marker of the ability to make 2-Me hopanoids.



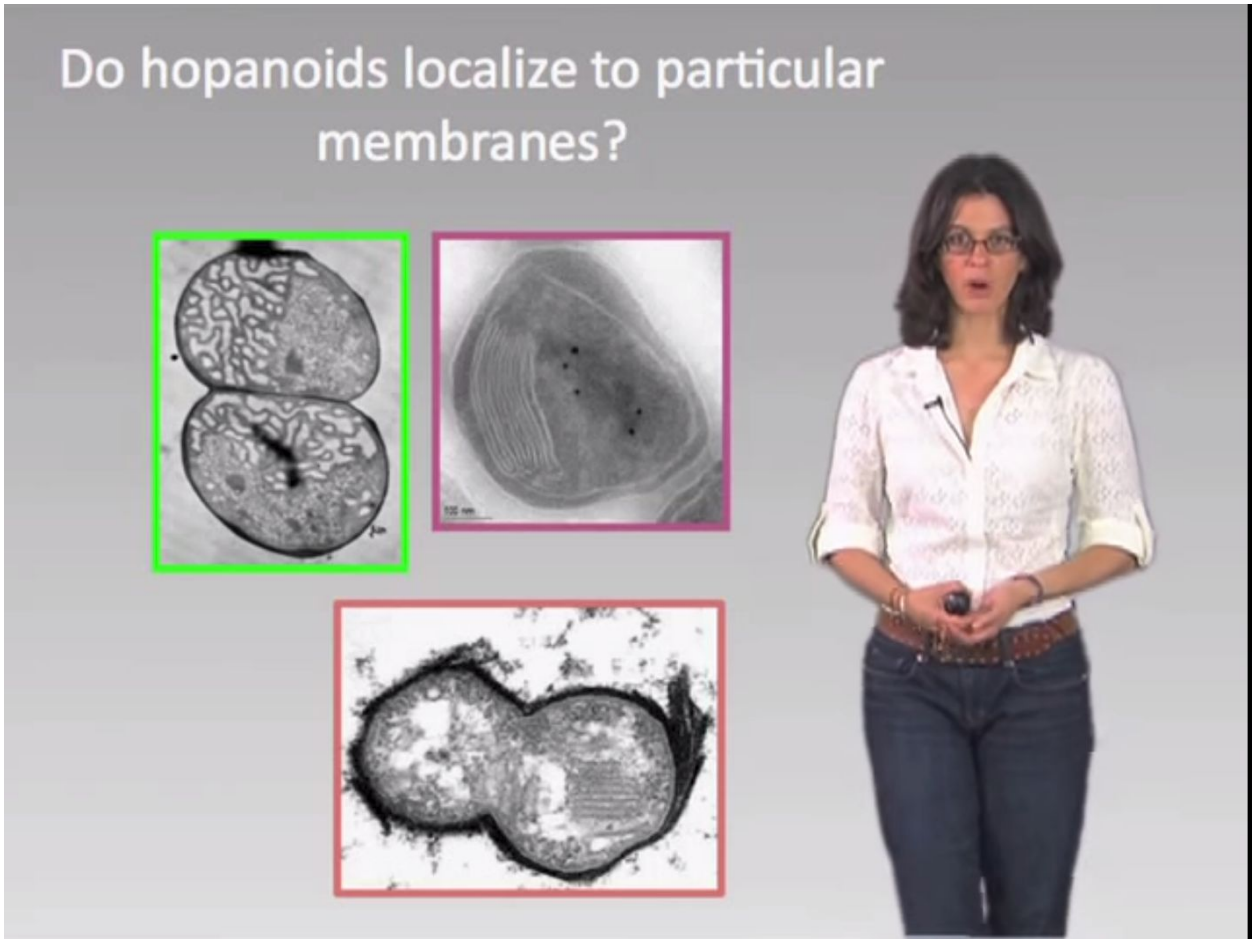
hpnP was found in non-cyanobacterial extant organisms.
However, 2-Me hopanoids could still be a good biomarker of cyanobacterial evolution if strong evidence could be found that the hpnP evolutionary root is in cyanobacteria.
Using bioinformatics to explore the phylogenetic history of the hpnP tree revealed that its origin is very ambiguous.
Even after varying many parameters of the hpnP phylogenetic tree construction, the root is still unclear and its identity is the subject of current research.



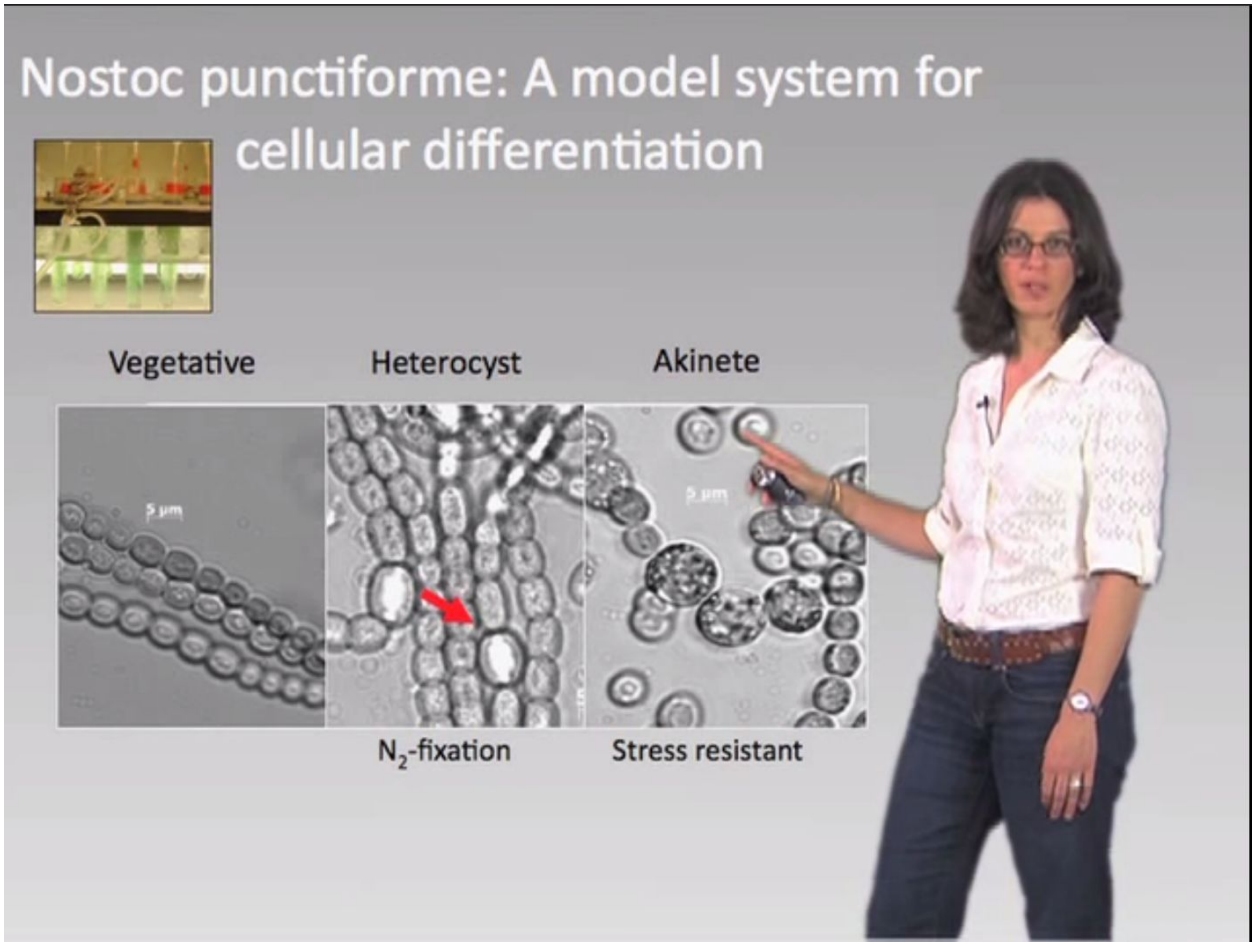
To further explore their potential role as biomarkers, the Newman lab asked whether 2-Me hopanoids have a specific and conserved biological function related to oxygenic photosynthesis.

Hopanoids closely resemble cholesterol. In eukaryotic membranes, sterols function in membrane fluidity and integrity, protein localization, and membrane curvature.

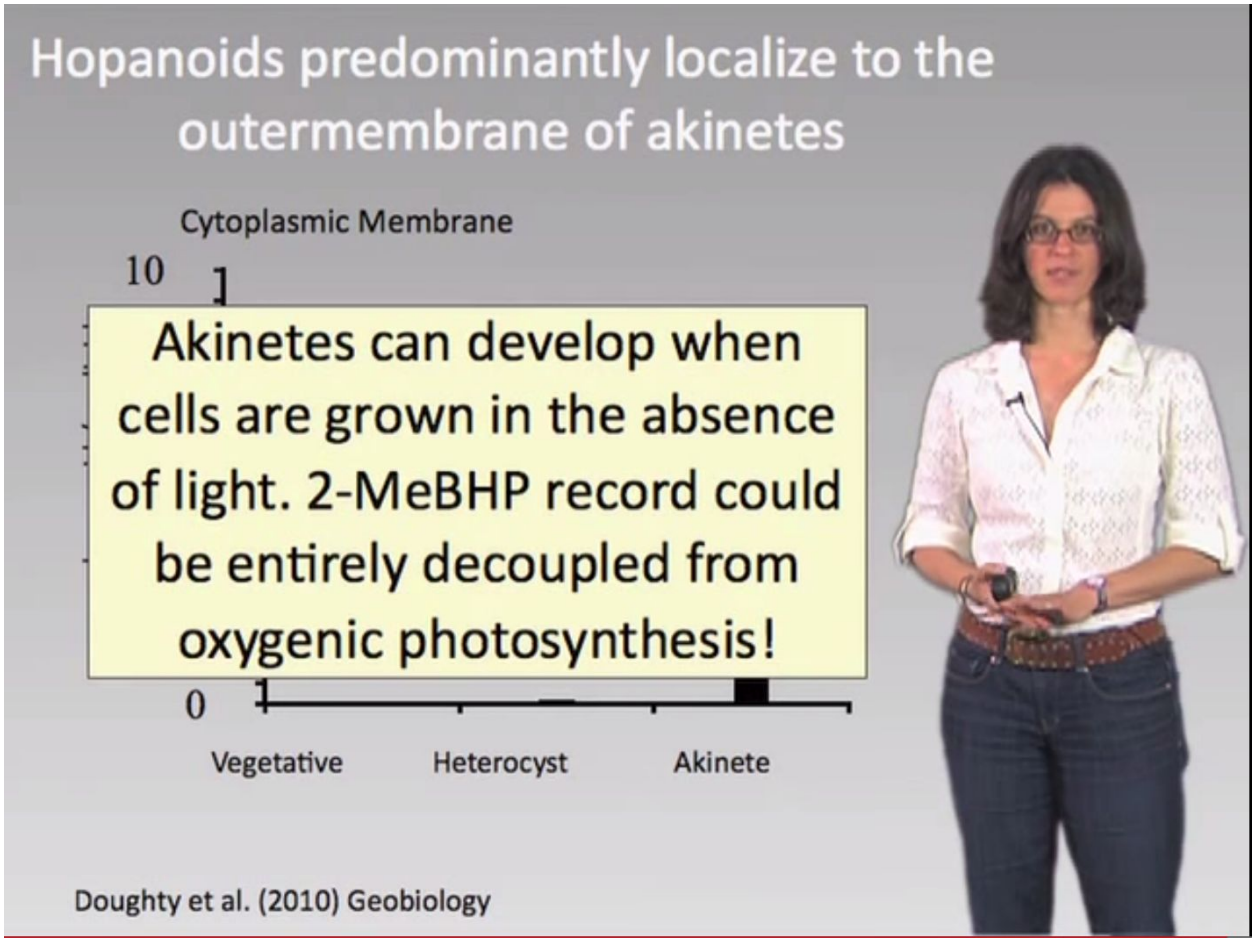
To determine whether 2-Me hopanoids may have played an essential role in the development of oxygenic photosynthesis, the researchers asked whether these compounds play similar roles as their sterol “cousins.”



Sterols influence membrane characteristics and oxygenic photosynthesis occurs in a particular membrane (thylakoid) compartment.
Therefore, the Newman lab asked whether hopanoids, in particular 2-Me hopanoids, localize to particular membranes.
Because microbial membranes are very complex, it was not initially clear whether hopanoids were specifically distributed.
To get a better sense of hopanoid membrane localization they utilized the Nostoc punctiforme model system, which reorganizes its membranes in 3 distinct cellular states.



The Newman lab asked whether hopanoid content and localization changes according to Nostoc punctiforme cellular state.
They found that hopanoids predominantly localize to the outer membrane in akinete cells.
Akinetes can develop when Nostoc cells are grown in the absence of light, which indicates that the 2-Me hopanoid fossil record could be entirely decoupled from oxygenic photosynthesis.
The Newman lab has theorized that the 2-Me hopanoid fossil record was produced by shedding of the akinete envelope into the sediments reflecting a stress response, as opposed to anything to do with oxygenic photosynthesis.



In summary, 2-Me hopanoids are produced by diverse microorganisms and it cannot yet be said which type of organism “invented” the ability to make 2-Me hopanoids.
Until the evolutionary root of hpnP is determined, it is unclear whether 2-Me hopanoids can be used as cyanobacteria biomarkers.
Cellular localization and other functional studies suggest that 2-Me hopanoids play no role in oxygenic photosynthesis.
The Newman lab’s work provides a clear example of how studies of modern bacteria can be used to investigate the evolution of metabolism.

3. Review Questions

1. What two processes convert organic molecules into molecular fossil biomarkers?
2. What type of organism is believed to have “invented” oxygenic photosynthesis?

3. Hopanoids are to bacteria as _____ are to eukarya?

4. Which of the following are Dr. Newman's criteria for a robust biomarker?

a. It must be indigenous to the rocks it is meant to represent.

    b. It must have a unique distribution amongst modern organisms, or clear evolutionary history.
    c. It must have a specific biological function related to the process of interest.
    d. All of the above.

5. Based on the phylogenetic analysis presented by Dr. Newman, which type of organism "invented" the ability to make 2-Me hopanoids?

6. Do 2-Me hopanoids have a unique distribution amongst modern organisms?

4. Answers to Review Questions

1. Sedimentation and diagenesis

2. Cyanobacteria

3. Sterols

4. d. All of the above

5. Even after the phylogenetic analysis, the organismal origin of 2-Me hopanoid synthesis was still unclear.

6. No, 2-Me hopanoids are produced by a phylogenetically-diverse group of modern organisms.

5. Discussion Questions

1. How does the story about *R. palustri*s emphasize the importance of running experiments under varied conditions?

2. If 2-Me hopanoids are not unique to cyanobacteria, then why does Dr. Newman suggest that 2-Me hopanoids could still be a biomarker of the origin of cyanobacterial photosynthesis?

3. Is there a direct connection between the ability to produce 2-Me hopanoids and oxygenic photosynthesis?
4. Why is it of interest to identify a specific role for 2Me-hopanoids in living cells?

6. Answers to Discussion Questions

1. Following the claim that 2-Me hopanoids can be used as biomarkers for cyanobacteria, the Newman lab investigated whether 2-Me hopanoids have a unique distribution amongst modern organisms. After collecting environmental samples of diverse microorganisms, the Newman lab tested the strains' ability to make 2-Me hopanoids. They included an alphaproteobacteria (non-cyanobacteria) *R. palustris* in their strain as a negative control, because this microorganism had been found to not produce hopanoids in previous studies. Only after growing the microbial samples under various growth conditions did the Newman lab discover that *R. palustris* was capable of producing 2-Me hopanoids in a growth condition-dependent manner. If the Newman lab had followed previous protocols and only looked at a narrow range of growth conditions, then they may have also incorrectly concluded that 2-Me hopanoids can be used as biomarkers for cyanobacteria. This story shows how important it is to run the same experiment in various conditions, because it is highly unlikely that a single condition will properly reflect the natural phenomenon.
2. If 2-Me hopanoids are not unique to cyanobacteria amongst extant microorganisms, phylogenetic analysis could still support the claim that 2-Me hopanoids are a biomarker of the origin of cyanobacterial photosynthesis. While today microorganisms other than cyanobacteria are capable of generating 2-Me hopanoids, it is currently unknown whether at some point in history only cyanobacteria were able to produce 2-Me hopanoids. The Newman lab showed that the ability to make 2-Me hopanoids is 100% correlated with the presence of the hpnP gene in all tested microorganisms. Therefore, if phylogenetic analysis reveals that the root of the hpnP phylogenetic tree is cyanobacterial, then it will indicate that 2-Me hopanoids are a biomarker of the origin of cyanobacterial photosynthesis. However, if it is determined that hpnP originated not in cyanobacteria, then it can be concluded that hpnP, and correspondingly 2-Me hopanoids, are not viable cyanobacterial photosynthesis biomarkers.
3. The most direction possible connection between the ability to produce 2-Me hopanoids and oxygenic photosynthesis would be the finding that 2-Me hopanoids

have a specific and conserved biological function related to oxygenic photosynthesis. As the function of 2-Me hopanoids is yet unknown, researchers have looked to their molecular ‘cousins’ eukaryotic sterols for potential functional roles. The comparison between hopanoids and sterols led researchers to look at the localization of hopanoids in particular microbial membranes, because sterols are known to play many roles in eukaryotic membranes. The Newman lab looked at the localization of hopanoids in the membranes of Nostoc punctiforme, a model system that exists in 3 cellular states each with very different membrane architecture. They found that hopanoids predominantly localized to the outer membrane in akinete cells, the stress-resistant form of Nostoc. Interestingly, akinetes can develop when cells are grown in the absence of light, which provides evidence that 2-Me hopanoid fossil record could be entirely decoupled from oxygenic photosynthesis. In summary, so far no one has established a direct connection between the ability to produce 2-Me hopanoids and oxygenic photosynthesis.

4. Hopanoid molecular fossils, ‘hopanes’, are incredibly geostable and present in sedimentary rock in almost unparalleled abundance. Molecular fossils, including hopanes, have long been recognized to hold information about the early history of life and past environments. 2-methylated hopanes have recently captured researchers attention, because a recent study suggested that 2-Me-hopanes were biomarkers of cyanobacteria, and therefore the process of oxygenic photosynthesis. To fully evaluate the claim that 2-Me-hopanes are biomarkers of cyanobacteria, it is necessary to identify the specific biological function for 2-Me-hopanoids in cells living today. Inferring a specific in vivo function for 2-Me-hopanoids would greatly improve researchers’ ability to interpret the claim that 2-Me-hopanes are biomarkers of cyanobacteria, as well as interrogate the 2-Me-hopane fossil record in general.

7. Explain or Teach These Concepts to a Friend

1. Explain the concept of knockout and rescue studies
2. Explain what it means to be a biomarker

8. Questions for Discussion Paper

Discussion Paper:

For the paper: Wu, C.-H., Bialecka-Fornal, M., and Newman, D.K. (2015). Methylation at the C-2 position of hopanoids increases rigidity in native bacterial membranes. Elife: 4:e05663.

1. How might the 2-methylation of hopanoids permit adventitious adaptation?

2. Why are the findings of previous hopanoid studies not likely to reflect the true roles of hopanoids in vivo?

3. What evidence suggests that methylation at the C-2 position of hopanoids promotes fitness under environmental stress?

4. What are the challenges to interpreting the meaning of any ancient molecular fossil?

9. Answers to Questions for Discussion Paper

1. For 2-methylation of hopanoids to permit adventitious adaptation, the modification must confer some sort of selective advantage to the host organism. One possibility is that 2-methylation of hopanoids provides biosynthetic precursors for further modification into novel components for signaling pathways. The likelihood of such a scenario is supported by the fact that sterols, which are often seen as the eukaryotic hopanoid analogues, serve as biosynthetic precursors for steroid hormones that play important signaling roles. Another possibility is that 2-methylated hopanoids may fulfill the geometry requirements of curve membranes and facilitate cell division. In this case, 2-methylation would clearly give a selective advantage because it would allow for more rapid and effective cell division. Given our still fairly limited knowledge of the effects of 2-methylation of hopanoids, it is conceivable that 2-Me-hopanoids bestow selective advantage through an entirely unanticipated mechanism.

2. Previous biophysical studies have provided insights into the functions of hopanoids, but it is unclear how relevant their findings are. Prior studies utilized model membranes as the context within which to study the role of hopanoids and the effect of 2- methylation. The lipid compositions and concentrations used in previous studies have not been consistent and do not necessarily provide a good model of in vivo cellular systems. If it is the case that the effect of hopanoid 2-methylation depends on membrane context, which is suggested by the findings of the Wu et al. paper, then it would be necessary for in vitro studies to mimic cellular composition as closely as possible. Furthermore, sterols, the eukaryotic hopanoid equivalent, are

known to differently modulate membranes depending on their lipid context. This indicates that in vitro studies will only be able to shed light on the in vivo role of hopanoid 2-methylation if there are able to fully replicate the relevant in vivo lipid environments.

3. Prior to the Wu et al. paper, there was already multiple lines of evidence suggesting that 2-Me-hopanoids promote fitness under environmental stress. 2-Me-hopanoids are enriched in the outer membrane of akinetes, the stress resistant cell type of the cyanobacterium Nostoc punctiforme. A stress-responsive pathway has been shown to upregulate the HpnP methylase in *R. palustris*, and this enzyme is responsible for methylating hopanoids at the C-2 position. Spikes in the 2-Me-hopane index (ratio of methylated hopanes to total hopanes) throughout time are correlated with oceanic anoxic events, which signify environmental stress. Lastly, in modern environments, the capacity for 2-Me-hopanoid production correlates with organisms, metabolisms, and environments that favor symbiotic plant-microbe interactions. The finding of Wu et al. that hopanoids differentially modify native membrane rigidity as a function of their methylation state supports the interpretation that 2-Me-hopane levels in fossils may reflect episodes of particular environmental stresses.

4. Molecular fossils hold rich details about early life, past environments, and evolution, but precautions must be taken in their interpretation. For a given molecular fossil to be interpreted, at least three criteria must be met. First, potential ancient sources for the fossilized compounds must be identified, with the necessity for unambiguous chemical parity between modern and ancient sources. Second, it is important to understand whether particular environmental conditions regulate the production of variants of the molecular fossil. Finally, it is critical to identify specific biological functions for the modern counterparts of molecular fossils, and then to evaluate whether the role of the molecular class has been conserved through evolution.