

## 147. Fluorescent probe selective for hydrogen atom abstraction based on polyethylene glycol cleavage

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### Project Goals:

**Problem.** Few organisms can break down lignocellulose, the primary structural component of terrestrial plants, and those with this ability employ unique, efficient biochemical mechanisms to deconstruct this polymeric composite. These mechanisms are relevant to US national energy needs because they may inspire the design of new technologies for biofuels production from abundant forms of lignocellulosic biomass such as wood. Research has shown that the only efficient lignocellulose degraders in nature are certain filamentous fungi that inhabit plant litter and wood, and that these fungi initiate lignocellulose deconstruction by disrupting the lignin that otherwise serves as a barrier to enzymes that can release the substrate's energy-rich sugars. There is also evidence that the agents responsible for lignin disruption are small molecules that oxidize this recalcitrant polymer. However, little is known about the specific identity of these oxidants, their quantity, or the processes that fungi use to generate them.

**Approach.** This project has the following goals and methods:

- 1) Develop new chemical sensors that resemble lignin structurally and are immobilized on small micrometer-scale beads that can be added to specimens of wood. When these sensors encounter a lignin-degrading oxidant, they will be cleaved to produce a fluorescent moiety that can be detected by fluorescence microscopy.
- 2) Develop methods to grow lignocellulose-degrading fungi on wood specimens that contain sensor beads.
- 3) Use fluorescence microscopy, in conjunction with these sensors, to quantify oxidants and obtain spatial maps of their distribution in wood undergoing deconstruction by several fungi whose genomes have been sequenced under the auspices of the US Department of Energy because they are candidates for bioenergy research. (1)
- 4) Develop mathematical models that account for the extents and spatial distributions of lignin-degrading oxidants in the biodegrading lignocellulosic specimens. This approach is expected to reveal which fungi employ the most efficient deconstruction mechanisms. (2)
- 5) Identify likely processes for lignin disruption by correlating the expression of fungal genes that may encode oxidant production with the oxidation of bead-linked sensors in the wood. This approach is expected to pinpoint fungal enzymes and metabolites that have key roles in disruption of the lignin barrier.

**Impact.** This research on biological processes involved in the efficient natural deconstruction of lignocellulose is likely to identify fungal enzymes and metabolites that have potential utility in biofuels development.

### Abstract

In nature, basidiomycete fungi are the major agents of lignocellulose recycling, using enzymes to produce reactive oxygen species that in many cases operate by hydrogen atom abstraction. In an attempt to understand the natural deconstruction of lignocellulose, we have developed a probe specific for this reaction. This probe carries two fluorescent dyes linked by a short polyethylene glycol (PEG) chain. Fluorescence FRET between these two dyes is lost when the PEG is depolymerized by a hydrogen-abstracting oxidant. That is, cleavage is observable by a loss of FRET between a fluorescent donor and acceptor on either end of the PEG molecule. The activity of these strong hydrogen abstracting species can thus be mapped by visualizing the loss of FRET. This work presents data on the basic structure of the probe and its response to various oxidants, along with preliminary data on its ability to report oxidative activity in real culture systems.

We have shown by NMR analysis that PEG is cleaved by H-abstraction oxidants. To capitalize on this observation, we have placed fluorescein as a FRET donor and TAMRA as an acceptor on either end of a PEG with a degree of polymerization of 7, which separates the dyes at most by 4.2 nm, which is within the FRET radius of this pair. We have further shown that FRET occurs in solution in this system and that oxidation reduces the occurrence of FRET, resulting in an increase in the donor signal relative to the acceptor. Finally, we have preliminary data showing a decline in FRET after this probe was placed on wood and exposed to the white rot fungus *Phanerochaete chrysosporium* for eight days. These results suggest that our system is capable of reporting oxidative activity on real lignocellulosic substrates.

Areas of improvement that we will explore for oxidation probes of this type are better pH stability, photostability and oxidation stability, as well as better solubility and a longer FRET radius for the donor and acceptor fluorophores. We are also attempting to anchor the probe to a solid substrate (3- $\mu$ m porous silica beads) to prevent diffusion of the probe or its ingestion by the fungi, which should allow more reliable measurements of the spatial distribution of oxidants on lignocellulose.

## References

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2. Hunt, C. G.; Houtman, C. J.; Jones, D. C.; Kitin, P.; Korripally, P.; Hammel, K. E. 2013, Spatial mapping of extracellular oxidant production by a white rot basidiomycete on wood reveals details of ligninolytic mechanism. *Environmental Microbiology* 15: 956-966.

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