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February 24, 1997

Gregory L. Dilworth  
Division of Energy Biosciences  
Office of Basic Energy Sciences, ER-17  
Department of Energy  
19901 Germantown Road  
Germantown, MD 20874-1290

Dear Dr. Dilworth:

This letter constitutes the final report for my DOE grant DE-FG06-92ER20093, "Biochemical Genetics of Lignin Degradation by *Phanerochaete chrysosporium*." During the years 1992-1996 we published 31 papers describing research which was funded at least in part by this grant or our previous DOE grant. Since this is a prodigious amount of work, it will be summarized briefly by category. The summaries and the emanating publications follow:

- I. **Peroxidase gene sequences:** We published new genomic sequences for lignin and Mn peroxidases (LiP and MnP).
- II. **Molecular biology studies on Mn Peroxidase and on *P. chrysosporium*:** We published four papers on the regulation of Mn peroxidase expression by various environmental signals including heat shock and oxidative stress. We also developed the first reporter system for studying the regulation of Mn peroxidase expression. Finally, we published papers on gene disruption in *P. chrysosporium* and on the isolation and transformation of uracil mutants of *P. chrysosporium*. In addition, we published a major review on the molecular biology of *P. chrysosporium*.
- III. **Crystal structures of fungal peroxidases:** We published the first papers on the crystal structures of both Mn and lignin peroxidases. These four papers represent a landmark in lignin degradation research.

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- IV. **Aromatic pollutant degradation by *P. chrysosporium*:** During this period we published four papers on the degradation of chlorinated and nitroaromatic pollutants by *P. chrysosporium*. These pollutants include chlorinated phenols and dibenzodioxins and nitroaromatic munitions.
- V. **Mechanisms of Mn and lignin peroxidase:** We published 5 papers on the mechanism of LiP and MnP. These papers concerned the oxidation of lignin models compounds and lignin model polymers by LiP as well as the mechanism of Mn oxidation by MnP.
- VI. **Expression and site directed mutagenesis of MnP:** We developed an homologous expression system for MnP and published our analysis of the first mutants of the Mn binding site of MnP.
- VII. **Intracellular enzymes involved in lignin and pollutant degradation:** We published papers on a quinone reductase, a nitroaromatic reductase and an aromatic ring opening dioxygenase from *P. chrysosporium*. We believe that all of these intracellular enzymes are involved in lignin and aromatic pollutant degradation.

**List of Publications on Research Supported by This Grant, By Category:**

**I. Peroxidase gene sequences**

A Highly Expressed Lignin Peroxidase Gene from the Basidiomycete *Phanerochaete chrysosporium*. T. G. Ritch, Jr. and M. H. Gold. *Gene* 118, 73-80 (1992).

Characterization of the *mnp2* Gene Encoding Manganese Peroxidase Isozyme 2 from the Basidiomycete *Phanerochaete chrysosporium*. M. B. Mayfield, B. J. Godfrey, and M. H. Gold. *Gene* 142, 231-235 (1994).

**II. Molecular biology studies on Mn Peroxidase and on *P. chrysosporium***

Molecular Biology of the Lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. M. H. Gold and M. Alic. *Microbiol. Rev.* 57, 605-622 (1993).

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Isolation and Transformation of Uracil Auxotrophs of the Lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. L. Akileswaran, M. Alic, E. K. Clark, J. L. Hornick, and M. H. Gold. Curr. Genet. 23, 351-356 (1993).

- Gene Replacement in the Lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. M. Alic, L. Akileswaran, and M. H. Gold. Gene 136, 307-311 (1993).

Heat Shock Induction of Manganese Peroxidase Gene Transcription in *Phanerochaete chrysosporium*. J. A. Brown, D. Li, M. Alic, and M. H. Gold. Appl. Environ. Microbiol. 59, 4295-4299 (1993).

A Reporter Gene Construct for Studying the Regulation of Manganese Peroxidase Gene Expression. B. J. Godfrey, L. Akileswaran, and M. H. Gold. Appl. Environ. Microbiol. 60, 1353-1358 (1994).

Nitrogen Regulation of Lignin Peroxidase Gene Transcription. D. Li, M. Alic, and M. H. Gold. Appl. Environ. Microbiol. 60, 3447-3449 (1994).

Regulation of Manganese Peroxidase Gene Transcription by Hydrogen Peroxide, Chemical Stress and Molecular Oxygen. D. Li, M. Alic, J. A. Brown, and M. H. Gold. Appl. Environ. Microbiol. 61, 341-345 (1995).

### III. Crystal structures of fungal peroxidases

Crystal Structure of Lignin Peroxidase. S. L. Edwards, R. Raag, H. Wariishi, M. H. Gold, and T. L. Poulos. Proc. Natl. Acad. Sci. USA 90, 750-754 (1993).

Crystallographic Refinement of Lignin Peroxidase at 2 Å. T. L. Poulos, S. Edwards, H. Wariishi, and M. H. Gold. J. Biol. Chem. 268, 4429-4440 (1993).

Preliminary Crystallographic Analysis of Manganese Peroxidase from *Phanerochaete chrysosporium*. M. Sundaramoorthy, K. Kishi, M. H. Gold, and T. L. Poulos. J. Mol. Biol. 238, 845-848 (1994).

The Crystal Structure of Manganese Peroxidase from *Phanerochaete chrysosporium* at 2.06 Å Resolution. M. Sundaramoorthy, K. Kishi, M. H. Gold, and T. L. Poulos. J. Biol. Chem. 269, 32759-32767 (1994).

#### IV. Aromatic pollutant degradation by *P. chrysosporium*

Degradation of 2,4-Dinitrotoluene by the Lignin-degrading Fungus *Phanerochaete chrysosporium*. K. Valli, B. J. Brock, D. Joshi, and M. H. Gold. *Appl. Environ. Microbiol.* 58, 221-228 (1992).

Degradation of 2,7 Dichlorodibenzo-*p*-dioxin by the Lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. K. Valli, H. Wariishi, and M. H. Gold. *J. Bacteriol.* 174 2131-2137 (1992).

Degradation of 2,4,5-Trichlorophenol by the Lignin-degrading Basidiomycete *Phanerochaete chrysosporium*. D. K. Joshi and M. H. Gold. *Appl. Environ. Microbiol.* 59, 1779-1785 (1993).

Oxidation of Dibenzo-*p*-dioxin by Lignin Peroxidase from the Basidiomycete *Phanerochaete chrysosporium*. D. K. Joshi and M. H. Gold. *Biochemistry* 33, 10969-10976 (1994).

#### V. Mechanisms of Mn and lignin peroxidase

Oxidation of Phenolic Arylglycerol- $\beta$ -aryl Ether Lignin Model Compounds by Manganese Peroxidase from *Phanerochaete chrysosporium*: Oxidative Cleavage of an  $\alpha$ -Carbonyl Model Compound. U. Tuor, H. Wariishi, H. E. Schoemaker, and M. H. Gold. *Biochemistry* 31, 4986-4995 (1992).

Mn<sup>II</sup> Oxidation by Manganese Peroxidase from *Phanerochaete chrysosporium*: Kinetic Mechanisms and Role of Chelators. H. Wariishi, K. Valli, and M. H. Gold. *J. Biol. Chem.* 267, 23688-23695 (1992).

Recent Studies on Lignin and Manganese Peroxidase from the Basidiomycete *Phanerochaete chrysosporium*. M. H. Gold, H. Wariishi, M. B. Mayfield, and K. Kishi. In: *Plant Peroxidases: Biochemistry and Physiology* (K. G. Welinder, S. K. Rasmussen, C. Penel, and H. Greppin, Eds.), University of Geneva, pp. 87-95 (1993).

Mechanism of Manganese Peroxidase Compound II Reduction: Effect of Organic Acid Chelators. K. Kishi, H. Wariishi, L. Marquez, H. B. Dunford, and M. H. Gold. *Biochemistry* 33, 8694-8701 (1994).

- Oxidation of Cytochrome *c* by Lignin Peroxidase. H. Wariishi, D. Sheng, and M. H. Gold. *Biochemistry* 33, 5545-5552 (1994).
- Oxidation of Dimethoxylated Aromatic Compounds by Lignin Peroxidase of *Phanerochaete chrysosporium*. D. Joshi and M. H. Gold. *Eur. J. Biochem.* 237, 45-57 (1996).

Purification and Characterization of Two Manganese Peroxidase Isozymes from the White-Rot Basidiomycete *Dichomitus squalens*. F. H. Périé, D. Sheng, and M. H. Gold. *Biochim. Biophys. Acta* 1297, 139-148 (1996).

## VI. Expression and site directed mutagenesis of MnP

Homologous Expression of Recombinant Manganese Peroxidase in *Phanerochaete chrysosporium*. M. B. Mayfield, K. Kishi, M. Alic, and M. H. Gold. *Appl. Environ. Microbiol.* 60, 4303-4309 (1994).

The Manganese Binding Site of Manganese Peroxidase: Characterization of an Asp179Asn Site-Directed Mutant Protein. M. Kusters-van Someren, K. Kishi, T. Lundell, and M. H. Gold. *Biochemistry* 34, 10620-10626 (1995).

Characterization of Mn<sup>II</sup> Binding Site Mutants of Manganese Peroxidase. K. Kishi, M. Kusters-van Someren, M. B. Mayfield, J. Sun, T. M. Loehr, and M. H. Gold. *Biochemistry* 35, 8986-8994 (1996).

## VII. Intracellular enzymes involved in lignin and pollutant degradation

Purification and Characterization of a 1,2,4-Trihydroxybenzene 1,2-Dioxygenase from the Basidiomycete *Phanerochaete chrysosporium*. S. Rieble, D. K. Joshi, and M. H. Gold. *J. Bacteriol.* 176, 4838-4844 (1994).

Aromatic Nitroreductase from the Basidiomycete *Phanerochaete chrysosporium*. S. Rieble, D. K. Joshi, and M. H. Gold. *Biochem. Biophys. Res. Commun.* 205, 298-304 (1994).

Purification and Characterization of a 1,4-Benzoquinone Reductase from the Basidiomycete *Phanerochaete chrysosporium*. B. J. Brock, S. Rieble, and M. H. Gold. *Appl. Environ. Microbiol.* 61, 3076-3081 (1995).

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1,4-Benzoquinone Reductase from the Basidiomycete *Phanerochaete chrysosporium*: Spectral and Kinetic Analysis. B. J. Brock and M. H. Gold.  
Arch. Biochem. Biophys. 331, 31-40 (1996).

**Budget:**

All of the funds for the four years of this grant have been expended.

Thank you for your generous support of our work.

Sincerely,



Michael H. Gold  
Institute Professor

MHG:nc