UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BAYER CROPSCIENCE LP, MONSANTO COMPANY, AND

INARI AGRICULTURE, INC.

Petitioners,

v.

CORTEVA AGRISCIENCE LLC,
Patent Owner.

IPR2023-01036
Patent 10,947,555

DECLARATION OF J. MARTIN BOLLINGER, JR., Ph.D.

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I, J. Martin Bollinger, Jr., hereby declare as follows:

I. Introduction

1. I have been retained by Finnegan, Henderson, Farabow, Garrett & Dunner, LLP on behalf of Patent Owner Corteva Agriscience LLC as an independent expert in the field of protein biochemistry, including enzymology and enzyme variants. I am being compensated for the time I spend on this matter, but no part of my compensation depends on the outcome of this proceeding.

II. Professional Background and Qualifications

2. I am an enzymologist and the endowed Russell and Mildred Marker Professor of Natural Products Chemistry in the Department of Chemistry and the Department of Biochemistry and Molecular Biology at The Pennsylvania State University. I received my Ph.D. from the Massachusetts Institute of Technology (MIT) in 1993 and was an N.I.H.-sponsored postdoctoral fellow at Harvard Medical School from January 1993 through May 1995. I am a Fellow of the American Association for the Advancement of Science.

3. I have taught undergraduate courses in chemistry and biochemistry as well as graduate courses in the mechanisms of enzyme action. I have also lectured at numerous professional conferences, universities, and in corporate R&D laboratories on those topics.

4. I have expertise in the science of enzymes. In my research laboratory, we study enzymes involved in the production of DNA, natural-product drugs, and biofuels. Other enzymes that we investigate degrade natural and anthropogenic compounds to enable bacteria to acquire essential elements such as phosphorus and sulfur. Some of these enzymes, including the enzymes at issue in this case, are used in agricultural applications. I have consulted for several companies, including the biotechnology companies KinTek, LS9, Joule Unlimited, Dow AgroSciences (now Corteva), and the pharmaceutical company GlaxoSmithKline.

5. I have received several prizes and awards, including the Dreyfus Foundation New Faculty Award (1995), the Searle Scholar Award (1996), the International Society of Biological Inorganic Chemistry's Early Career Award (2008), Penn State University's Faculty Scholar Medal for Outstanding Achievement in the Physical Sciences (2009), Penn State's Howard Palmer Award for Faculty Mentoring (2012), Penn State's Distinguished Faculty Mentoring Award (2021), the American Society for Biochemistry and Molecular Biology's William C. Rose Award (2022), the American Chemical Society Division of Biological Chemistry's Abeles and Jencks Award (2023), and the American Chemical Society's Nobel Laureate Signature Award for Graduate Education in the Chemical Sciences (2023).

6. I have directed the research activities of 21 Ph.D. graduates, 7 M.S. graduates, and approximately 24 post-doctoral fellows; most of these trainees have gone on to successful careers in the chemical and biochemical industries or in academia, including positions at Stanford University, the University of Pennsylvania, the University of Washington (Seattle), Brigham Young University, Carnegie Mellon University, North Carolina State University, Brandeis University, and Penn State University.

7. I have delivered several named and plenary lectures at universities and scientific conferences, including the 2018 TY Shen Lecture in Biological Chemistry at M.I.T. and keynote lectures at the 2009 International Conference in Biological Inorganic Chemistry in Nagoya, Japan, the 2015 Southeast Enzymes Conference in Atlanta, GA, and the 2020 Midwest Enzyme Chemistry Conference (remote). In total, I have delivered more than 50 lectures at conferences, nearly 80 lectures at colleges and universities, and 6 lectures at companies.

8. I have co-chaired two international conferences (Gordon Research Conferences) and served as Program Chair of the American Chemical Society's Division of Biological Chemistry. I have also served as co-chair of a federally funded, biennial international training program in biological inorganic chemistry at Penn State.

9. I have served on the editorial advisory boards for the scientific journals *Journal of Biological Chemistry*, *Biochemistry*, and *Journal of Biological Inorganic Chemistry*. I have also served as section editor of reference book series and special issues of journals, including *Comprehensive Natural Products* and *Current Opinion in Chemical Biology*.

10. I have published ~140 original scientific research articles as well as ~21 invited scientific review articles, book chapters, and perspectives. My research team specializes in elucidating the structures and mechanisms of enzymes that use transition metals to react with oxygen gas (O_2) in the atmosphere to transform carbon compounds. All but a few of the more than 160 publications noted above focus on such oxygen-utilizing metalloenzymes.

11. One of our most successful long-term projects (since 2002) concerns iron- and α-ketoglutarate-dependent oxygenases, which are at issue in this case. I have co-authored 40 publications focused exclusively on one or more of the enzymes in this large class. Three of these articles were published in the high-profile journals *Science* and *Nature*, and five others appeared in the top journals *Proceedings of the National Academy of Sciences of the United States of America* and *Nature Chemical Biology*. I have co-authored seven other articles that discuss these enzymes at least in part.

12. My *curriculum vitae* (CV) is attached as **Appendix A** and lists the litigations and other matters on which I have served as an expert witness. Also listed on my CV are books and articles I have co-authored, including peer-reviewed journal publications.

III. Materials Considered

13. My opinions are based on my education, training, background and experience, the documents I reviewed, and information available to me to date. I have reviewed and considered the documents and materials cited in this declaration, as well as those listed in **Appendix B**. To the extent I am provided with additional documents or information in this proceeding, I reserve the right to modify or expand upon my opinions based on such new information, and in response to any additional reports and testimony.

IV. Legal Standards

14. The opinions I express in this declaration involve the application of my technical knowledge and experience to evaluating certain prior art and other scientific and technical issues relating to the ’555 patent. In preparing this declaration, certain patent law concepts have been explained to me by counsel, including the legal standards for assessing written description and obviousness.

15. I understand that written description must be analyzed from the perspective of a “*skilled artisan*” (or “skilled person”) in the same field as the

challenged patent as of the "effective filing date" of the claims. I understand that a skilled artisan is presumed to know of the relevant art (e.g., technical literature) as of the "effective filing date" of the claims.

16. I understand that the "*effective filing date*" is evaluated on a claim-by-claim basis and that it is the filing date of the earliest priority application that provides "*written description*" for the subject matter of the claim. In this case, as I explain below, the relevant time period is in 2004-2005, when Corteva filed its initial patent applications that led to the '555 patent. Ex. 1021 (PCT/US2005/014737); Ex. 1004 (Provisional 60/567,052).

17. I understand that the basic inquiry for written description is whether the specification describes the claimed subject matter such that a skilled person would understand that the inventor possessed what is claimed. I understand that a number of factors may be considered in evaluating the written description requirement, including: (1) the disclosure of the specification; (2) the existing knowledge in the particular field; (3) the extent and content of the prior art; (4) the maturity of the science or technology; and (5) the predictability of the technical feature at issue. I have been instructed that a sufficient description of a genus (such as a group of enzymes) requires the disclosure of either a representative number of species or structural features common to the genus so that one skilled in the art can visualize or recognize the members of the genus.

18. I have also been asked to analyze whether the challenged claims of the ’555 patent would have been obvious to a person of ordinary skill in the art as of the earliest filing date (April 30, 2004). In conducting this analysis, I have considered the scope and content of the prior art, any differences between the claims and the prior art, the level of ordinary skill in the art, and objective evidence of nonobviousness (e.g., whether the invention claimed exhibits unexpected results). I have also considered whether a person of ordinary skill in the art would have had reasons or motivations to combine the prior art with a reasonable expectation of successfully achieving the claimed invention.

19. I understand that any obviousness analysis must not be driven by hindsight but must be based on the prior art as of the effective filing date. I understand that a patent claim must be evaluated as a whole, such that all claim limitations are considered together against the prior art.

V. Background and State of the Art

20. I provide a brief summary below of what was known in the prior art about enzyme structure-function correlations, including for αKG-dependent dioxygenases such as RdpA (AAD-1) and TfdA. This information would have been known to persons skilled in the art and generally accepted in 2004-2005.

A. Enzymes

1. Function, Structure, and Classification of Enzymes

21. Enzymes function as catalysts for biochemical reactions. Some enzymes catalyze reactions that would otherwise be too slow to be useful to cells and organisms. Without enzymes, many biochemical reactions would take much longer and would not be fast enough to sustain life. *See* Ex. 2018 (Robinson), 2. An enzyme catalyzes a chemical reaction by binding its substrate(s) (i.e., the chemical compounds required for the reaction) at a location within an interior pocket known as the active site. Ex. 2018 (Robinson), 4.

22. The vast majority of enzymes are proteins (also known as polypeptides), which are large molecules built from 20 different types of amino acids. Ex. 2018 (Robinson), 4. To form enzymes, these amino-acid building blocks are connected in a linear chain in a specific sequence. The sequence of amino acids in a polypeptide chain is called its primary structure. *See* Ex. 2018 (Robinson), 4. This linear chain then folds up into a "globular" (meaning more or less spherical) three-dimensional structure in order to function as an enzyme catalyst. The secondary structure of a protein describes the localized polypeptide chain structures such as α-helices and β-sheets. *See* Ex. 1023 (Elkins), 5186-87 (discussing β-sheets and α-helices). The complete three-dimensional fold of a polypeptide chain into a globular protein subunit is known as its tertiary structure. Proteins may be composed

of one or more subunits (e.g., a monomer, dimer, trimer, etc.). *See id*. at 5185, 5188. The three-dimensional arrangement of subunits in an enzyme is known as its quaternary structure.

23. Enzymes are classified using a specific enzyme nomenclature system known as an Enzyme Commission or "EC" number. This system was "the best developed and most widely used functional classification scheme" in 2004-2005. Ex. 2019 (Tian), 864. An EC number is a unique four-digit code that categorizes the chemical reaction that an enzyme catalyzes. Ex. 2019 (Tian), 864. The first digit of an EC number indicates the main type of enzymatic activity and ranges from 1 to 6[1], including: 1, oxidoreductases, 2, transferases, 3, hydrolases, 4, lyases, 5, isomerases, and 6, ligases. Ex. 2020 (Tipton), 36. The last digit usually represents the substrate specificity of a reaction, while the first three digits of the EC number usually describe the overall type of enzymatic reaction. Ex. 2020 (Tipton), 36-37.

24. An enzyme's structure and the reaction it catalyzes (its function) are usually determined by its sequence of amino acids or primary structure. *See* Ex. 2021 (Rost 1999), 85 ("proteins with similar sequences adopt similar structures"). Enzymes with similar sequences usually catalyze similar or identical reactions, as further described below.

[1] A new class of enzymes, the translocases (EC 7), was added in 2018.

2. Degrees of Amino Acid Sequence Relatedness

25. Skilled artisans have long analyzed the similarity of enzymes by comparing their amino acid sequences. Before 2004-2005, skilled artisans in the field had arrived at a general consensus for how to determine and describe the degree of relatedness of enzymes based on their amino acid sequences.

26. Skilled artisans perform sequence alignments to identify structural, functional, or evolutionary relationships between two or more DNA, RNA, or protein sequences. Nucleotides or amino acids (often referred to as residues) in a sequence are aligned to give the greatest possible number of matches between or among the sequences, which are typically represented as rows within a matrix. Gaps are inserted between the residues in individual sequences as necessary so that identical or similar residues are aligned in successive columns. *See, e.g.*, Ex. 1023 (Elkins), Fig. 5; Ex. 1042 (Schleinitz), Fig. 2.

27. By 2004-2005, researchers commonly performed sequence alignments to compare proteins of similar or dissimilar structure. Sequence identities (or similarities) between two or more enzymes may range from <10% (few matching residues) to 100% (identical). Ex. 1023 (Elkins), 5189 (identifying TfdA and TauD as "closely related proteins" based on ~30% sequence identity); Ex. 2021 (Rost 1999), 85. A sequence identity between two enzymes of above 35% or 40% is considered a relatively high level of sequence identity and indicates that those

enzymes likely have similar structures and functions. Ex. 2021 (Rost 1999), 85. Often, two enzymes with this level of sequence identity will catalyze the same reaction or similar reactions. Enzymes having less than 20% sequence identity generally have different secondary and tertiary structures and catalyze different types of reactions.

28. Decades of comparing and aligning protein sequences led researchers to recognize a "twilight zone" for sequence similarity. Ex. 2023 (Chung), 1123. This "twilight zone" represents a range of sequence identity (approximately 20-35%) in which the relatedness of proteins—how similar their structures are, whether they have the same function, and whether they evolved from a common precursor—can be more difficult to judge. Ex. 2023 (Chung), 1123; Ex. 2021 (Rost 1999), 85. In other words, relationships between sequence and function are often more unpredictable when sequence identities fall within the twilight zone, making enzymes in this sequence identity range particularly interesting for research purposes, with several groups spending significant time attempting to discern sequence/function relationships at different thresholds of sequence identity, including at and around the twilight zone of 20-35% sequence identity. Ex. 2021 (Rost 1999), 85; Ex. 2024 (Wilson), 245.

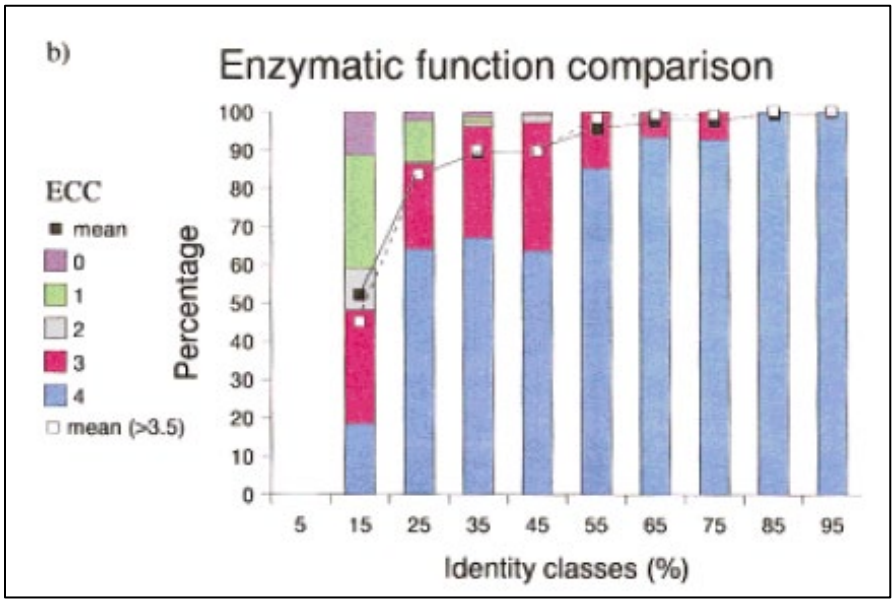
3. Predicting Enzyme Function Based on Sequence Identity

29. By the 2000s, genome sequencing was identifying new genes encoding proteins much faster than researchers could empirically determine the activities of those proteins. Researchers therefore sought to determine how reliably one could infer the function of a new protein by comparing its sequence to the sequence of another protein having a known function. Ex. 2025 (Minshull), 202 (two main reasons to predict an enzyme's function from its sequence are "to identify the components and thus the functional capabilities of an organism" and "to create enzymes with specific properties"). Using pairwise sequence comparisons, numerous studies have shown that when the sequence identity between enzymes is above 60%, enzyme function is well conserved. Ex. 2019 (Tian), 863; Ex. 2024 (Wilson), 233; Ex. 2026 (Devos), 98; Ex. 2027 (Todd), 1113; Ex. 2028 (Rost 2002), 595. This principle applied in 2004-2005 and remains true today. Ex. 2030 (Price), 1.

30. A study by Wilson et al. in 2000, for example, evaluated pairwise sequence, structure, and function comparisons on ~30,000 pairs of protein domains. Ex. 2024 (Wilson), 233. Wilson et al. constructed their own functional classification system using EC numbers for enzymes. Ex. 2024 (Wilson), 242. They found that full conservation of enzyme function can occur between two proteins with as low as ~40% sequence identity. Ex. 2024 (Wilson), 233. They concluded that percent

identity is a useful predictor of function for proteins that are close in structure and outside the twilight zone. Ex. 2024 (Wilson), 245.

31. A study in 2000 by Devos et al. analyzed a set of over 7,000 protein sequence alignments using the publicly available FSSP protein database. Ex. 2026 (Devos), 99. It found that, above an identity threshold of 50-80%, at least the first three digits of an EC number were identical, and above 80%, all four digits in the EC codes were "completely identical," meaning that two enzymes having this level of identity can confidently be assumed to have the same activity. Ex. 2026 (Devos), 101-102 (Fig. 1b, replicated below).



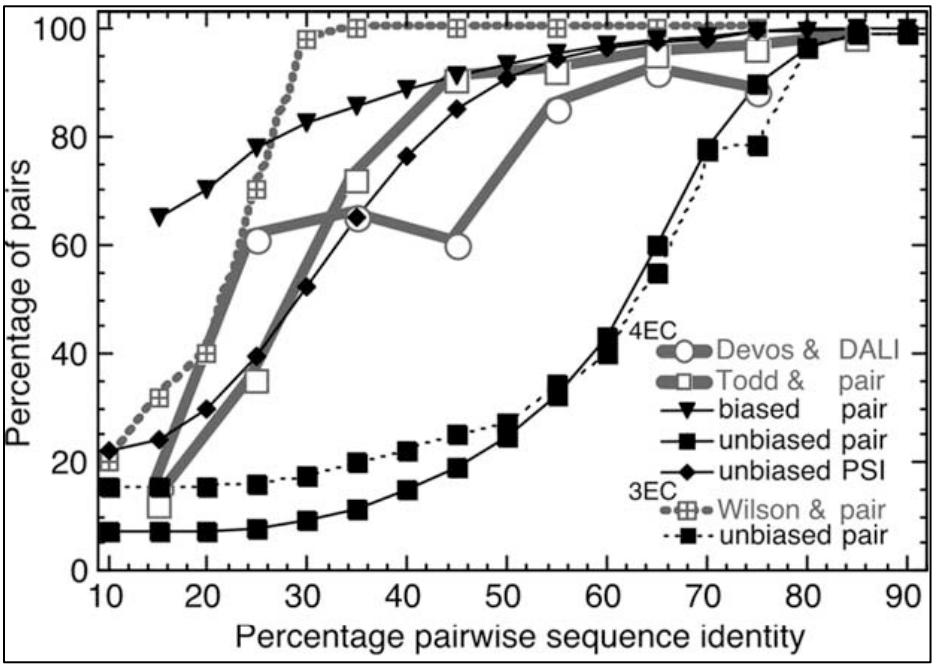
Ex. 2026 (Devos), 101 (Fig. 1b).

32. Similarly, a study by Todd et al. in 2001 assessed the functional variation of 31 diverse structural enzyme superfamilies using the EC classification scheme and found that functional variation is rare when the sequence identity is

above 40%. Ex. 2027 (Todd), 1113. Todd et al. also found that "conservation of chemistry does appear to be the dominant theme," further suggesting that high sequence identity between a pair of enzymes indicates that they will have the same biochemical function (i.e., catalyze the same reaction). Ex. 2027 (Todd), 1138.

33. In 2002, Rost made a more conservative estimate for sequence-based conservation of enzyme function aimed at providing "important benchmarks for everyday sequence analysis," suggesting that the prior analyses were either biased or too small. Ex. 2028 (Rost 2002), 595. Rost's analysis focused solely on enzymes with known function (each enzyme had an annotated EC number) from the curated SWISS-PROT database. Ex. 2028 (Rost 2002), 605. In this database, all enzymes were functional, and Rost further filtered the database to remove any enzymes whose function had yet to be fully characterized (i.e., having less than all 4 EC numbers) or where "more than one EC number was given (domains)." *Id*. Enzymes with multiple domains are known in the field as multi-enzymes, as they have multiple binding sites and can catalyze different types of reactions in their separate domains. *Id.* Using this functional data set, Rost considered sequence comparisons into the twilight zone and found that, when the sequence identity is below 70%, both the first digit and all four digits of EC numbers start to diverge more quickly than others had reported. Ex. 2028 (Rost 2002), 596; Ex. 2019 (Tian 2003), 863.

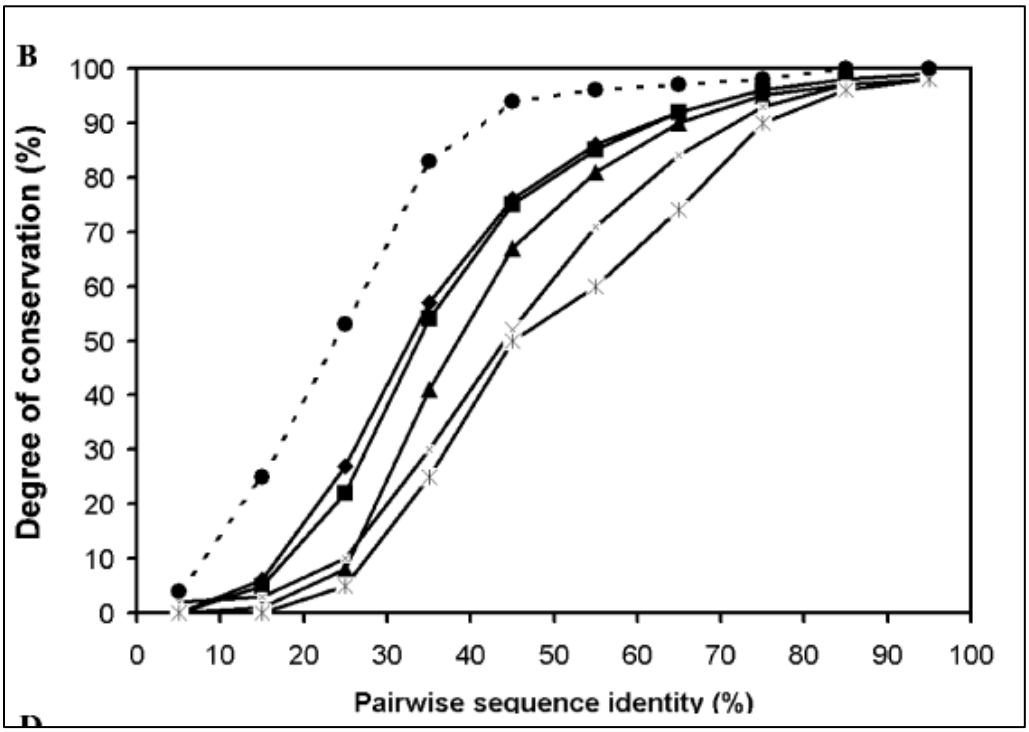
34. Nevertheless, Rost confirmed previous reports that enzyme function is highly conserved above 70% sequence identity. Ex. 2028 (Rost 2002), 596. Indeed, Rost presented a graphical comparison between his study and those by Wilson, Devos, and Todd, which confirmed an even stronger conservation of function at higher levels of sequence identity, including at 85% or greater identity:



Ex. 2028 (Rost 2002), 604 (Fig. 5).

35. A study by Tian et al. in 2003 consolidated and built upon the prior studies by Devos, Wilson, Todd, and Rost, analyzing thousands of protein sequences for establishing "the threshold of sequence identity necessary to transfer function from a protein of known function to an unknown protein." Ex. 2019 (Tian), 863. Using a strategy similar to Rost's to reduce database bias, Tian defined an enzyme family as a family of sequences that are all above a certain threshold of pairwise

sequence identity and that also have the same function. Ex. 2019 (Tian), 865, 876-77. Tian found that "40% sequence identity can still be used as a confident threshold to transfer the first three digits of an EC number," whereas "above 60% sequence identity is needed to have above 90% accuracy" for all four digits of an EC number. Ex. 2019 (Tian), 865.



Ex. 2019 (Tian), 866 (Fig. 1(B)). As shown in the figure, the x-axis represents percent sequence identity, and the y-axis represents percent function conservation.

36. Accordingly, by 2004-2005, persons skilled in the art routinely predicted enzyme function based on sequence similarity to proteins whose function had already been experimentally determined. The extensive studies detailed above showed that this was a universal principle applied within the field of enzymology.

See, e.g., Ex. 2026 (Devos), 98; Ex. 2019 (Tian), 864 ("sequence identity is [] widely used as an indication of functional similarity"). It was established by then that enzyme function is well conserved and can be reliably predicted based on sequence identities above approximately 50-60%. These established function prediction values were well below the 85% minimum threshold of the '555 patent claims. Ex. 2019 (Tian 2003), 863; Ex. 2026 (Devos), 102. In other words, there was a recognized trend that higher sequence identities correlated more strongly to conserved enzyme function than lower sequence identities, and 85% was a particularly high level of sequence identity from which skilled artisans would have expected the same enzyme function.

37. Indeed, sequence comparisons were the accepted "gold standard" for predicting protein function. Ex. 2029 (desJardins), 97; Ex. 2024 (Wilson), 233 ("percent identity is more effective at quantifying functional conservation" than other techniques); Ex. 2030 (Price), 1 ("Proteins with over 30% similarity are likely to have similar functions, but to be 90% confident of the substrate of an enzyme, over 60% similarity may be required[]") (citing Ex. 2019 (Tian), 863).

38. The strong correlation between a high level of enzyme sequence identity and conserved enzyme function was intuitive for skilled artisans in 2004-2005. Different enzymes that catalyze the same reaction were known generally to have significant sequence and structural similarity based on underlying sequence

conservation. Ex. 2032 (Galperin), 779-80. Structural analyses of enzymes having relatively high sequence identity have revealed recurrent structural patterns, such as common folds and conserved sequence motifs. Ex. 2032 (Galperin), 780. Persons skilled in the art recognized the evolutionary structural patterns of enzymes and often used their structures as a basis for inferring function for enzymes having related sequences.

39. By 2004-2005, skilled artisans also recognized amino acid sequence patterns—known as motifs—in enzymes to identify elements of their structures that are essential for their functions. Such motifs consist of individual amino acids or stretches of amino acids that are always identical in a set of enzymes with the same or similar functions but that can be interspersed by individual amino acids or stretches of amino acids (known as spacers) that differ among enzymes of the set. Ex. 1023, 5185, 5190 (Fig. 5). The always identical amino acids (referred to as "conserved" amino acids) within such a motif often have important functions in the common activity of the set of enzymes, such as in binding of substrates or other components (known as cofactors) that are required to promote the chemical reaction. Motifs are amino acid sequence patterns that are generally related to a specific function of the protein. Ex. 1042, 5360 (Fig. 2), 5357 (describing the motif residues in RdpA as "functionally important"). By 2004-2005, researchers had recognized that, when a set of enzymes has an overall level of sequence identity of about at least

60-70%, such sequence motif patterns that are fully conserved within the set of enzymes can readily help identify residues that are important to the common structure and function and, importantly, be used to assign new members to the set as new protein sequences are reported. Ex. 2033 (Dym), 1712. A motif is often one of the most reliable structural features for inferring common function between enzymes. Ex. 1035 (Müller 2006), 1358; Ex. 2034 (Minarik), 261 (explaining how "[r]esidue 53 is conserved with an acidic side chain in all NAD-dependent MDHs"). For instance, malate dehydrogenases are a class of enzymes that promote one common chemical reaction and use a conserved catalytic mechanism. Malate dehydrogenases from different species share a high degree of 3D (tertiary) structural similarity. Ex. 2034 (Minarik), 257. The common functional part of malate dehydrogenases—their catalytic domain—is characterized by a particular motif that is conserved throughout the class and contributes to both the conserved structure and the shared enzymatic activity. Ex. 2034 (Minarik), 259.

40. In sum, by 2004-2005, high similarity of enzyme amino acid sequences was known to correlate well with conservation of both overall three-dimensional structure and function, which enzymologists recognized as a general rule. Ex. 2032 (Galperin), 779. Thus, when an amino acid sequence appearing to represent a new enzyme was determined as part of the genome-sequencing efforts of the 1990s and 2000s, skilled artisans routinely assigned a biochemical function to the new enzyme

using sequence comparisons and motif recognition. These principles were well established and also routinely applied in protein engineering to make enzyme variants with conserved or even improved functional traits. *See* Section V.B, below; Ex. 2025 (Minshull), 202-3 ("Using homologous sequences for protein engineering").

4. Enzymes Are Unique Compared to Other Types of Proteins

41. Several categories of proteins occur in nature, including enzymes, antibodies, structural proteins, hormones, and others. Skilled artisans were familiar with differences between the various types of proteins and what made enzymes unique. Among these categories of proteins, enzymes and antibodies have been extensively used in medicine and biotechnology and thus have been the subjects of considerable scientific research.

42. The fundamental precept of sequence-based conservation of function in enzymology can be illustrated by the differences between enzymes and antibodies. One major difference between these types of proteins is their evolutionary background. Because enzymes catalyze biochemical reactions, many of which were likely important even to early life forms, they are generally products of evolution over long time periods (e.g., years). Even enzymes that catalyze the identical reaction can have quite different amino acid sequences as a result of the long time over which they evolved from their common ancestral enzyme. However, any amino

acid changes (mutations) appearing over time that disturbed the enzyme's overall structure or replaced amino acids that promoted the reaction would have led to a loss or reduction of the enzyme's activity, likely with negative consequences for the organism that depended on the enzyme. Accordingly, pairs of enzymes catalyzing the same reaction (isofunctional enzymes) have retained common, relatively optimized structures conserving important amino acids that are responsible for certain facets of the overall enzyme activity (e.g., structural stability, substrate binding, transition state stabilization). *See, e.g.*, Ex. 2019 (Tian), 877. Amino acids in a protein sequence that are less important to an optimized structure or catalytic function are replaced over time by different amino acids, with no deleterious effect on protein function. The retained portions are functional motifs, such as the AAD-1 motif at issue here. In other words, enzymes that have evolved over time to perform the same function (or similar functions) can span a relatively broad range of sequence space. *See, e.g.*, Ex. 2034 (Minarik), 258-59 (malate dehydrogenases have ~20% sequence similarity but have homologous active sites, coenzyme binding sites and quaternary structure).

43. Antibodies are starkly different proteins than enzymes. Antibodies develop rapidly over a matter of weeks as part of an immunological response to an antigen molecule, to which the antibody binds. Ex. 2037 (Wedege), 572-73 (antibodies detected 6 weeks post vaccination). Antibody structures and sequences

are evolutionarily adapted to this rapid evolution, as large portions of antibodies have generally fixed sequences (known as constant regions) while sequence changes leading to diversity in antigen recognition are quickly generated in smaller variable regions, particularly the hypervariable regions known as complementarity determining regions (CDRs). Ex. 2047 (Llewelyn), 1270. These variable regions are, on average, much shorter in sequence than enzymes. Thus, the sequence of antibodies can rapidly evolve in relatively discrete regions as part of an immunological response to an antigen, with small changes within the variable regions of an antibody often leading to enormous differences in function. In other words, the variable regions of antibodies, which generally determine their different functions, generally cannot be grouped into "buckets" of similar sequences, as with enzymes.

44. In addition, modifications in enzyme sequence tend to be located throughout the enzyme's amino acid sequence, unlike the focused tailoring of the variable and hypervariable regions of an antibody to change its binding activity and confer recognition of a new antigen. Ex. 2047 (Llewelyn), 1270. Because antibodies serve a different biological role, which requires rapid evolution of their structures to confer the ability to recognize new antigens, even small changes in variable region sequence can lead to entirely different antigen targets and therapeutic properties. The

same does not hold true for enzymes, which generally have conserved structures among enzymes having identical or similar functions.

B. Enzyme Engineering and Variants

45. In his declaration, Dr. Hausinger asserts the genus of AAD-1 enzymes of the ’555 patent claims is vast, consisting of at least 20^{44} proteins. Ex. 1054, ¶¶ 77-86. This assertion rests on the assumption that any of the 20 amino acids could be present at as many as 44 positions in the sequence of AAD-1. I disagree with Dr. Hausinger’s opinion, which appears to ignore common knowledge possessed by a skilled artisan in the field. When making enzyme variants, a person skilled in the art in 2004-2005 would not have randomly picked any of the 20 possible amino acids for a given residue but would have instead relied on known techniques and principles for generating enzyme variants.

1. Sequence Alignments

46. One important available technique was use of sequence alignments. Ex. 2025 (Minshull), 203 (“The sequences of orthologous proteins are thus a potentially rich source of information regarding sequence changes that are acceptable without drastically disrupting protein function….”).

47. By 2004-2005, alignment tools for comparing protein sequences were recognized and widely used by persons skilled in the art. These tools allowed researchers to compare two or more protein sequences and calculate percent identity

or similarity scores using known algorithms. These algorithms had been incorporated into various programs, such as BLAST (Basic Local Alignment Search Tool) and FASTA (FAST-All), which were publicly available and widely used by skilled artisans in 2004-2005. Ex. 2040 (Altschul), 3389; Ex. 2041 (Müller), 761; Ex. 1001 ('555 Pat.), 44:14-35.

48. Aligned sequences of amino acid residues are typically represented as rows within a matrix. Matching residues in the aligned sequences are typically marked using asterisks or pipe symbols. When the aligned sequences differ in length, gaps are inserted between the residues so that identical or similar residues can be aligned in successive columns.

49. By 2004-2005, sequence alignments were known to be annotated with structural information, including alpha helices and beta strands. While not mandatory, a skilled artisan would have recognized the value of structural information in a sequence alignment. Structural information is instructive to a skilled artisan in recognizing similarities between different enzyme family members, especially those in the twilight zone. An exemplary sequence alignment, which contains such structural annotation, is shown in Figure 5 of Elkins et al. Ex. 1023 (Elkins), 5190 (Fig. 5).

50. The quality of a possible alignment of two or more sequences is frequently assessed using a substitution matrix, which have been known since the

1970s and were automated and computer-implemented, for example with the SIFT (Sorting Intolerant From Tolerant) program, by 2004-2005. These matrices assign probabilities of seeing one of the 20 common amino acids replaced by another. One class of scoring matrix, given the acronym PAM (for Point Accepted Mutation), explicitly accounts for evolutionary approximations regarding the rates and probabilities of the possible amino acid substitutions. Ex. 2041 (Müller), 761. Another common scoring matrix, known as BLOSUM (Blocks Substitution Matrix), encodes empirically derived substitution probabilities. Ex. 2041 (Müller), 761. Results generated from either type of matrix allow skilled artisans to quantify the level of sequence relatedness or divergence. For example, in searching sequence databases to identify proteins related to an enzyme of interest (the query sequence), users of BLAST or FASTA can set threshold values either to restrict the identified sequence set (the "hits") to more closely related matches or to expand the set to include less similar (more divergent) sequences.

51. When interpreting aligned sequences, it was common for skilled artisans in 2004-2005 to examine the degree of conservation of the amino acids within a stretch of the sequence as a rough measure of how conserved a particular region is among lineages. In a multiple sequence alignment, positions that have the identical amino acid in all the aligned sequences are considered highly conserved, and, if the side chain of the amino acid found there has an appropriate chemical

functional group (such as the carboxylic acid group of Asp or Glu, the imidazole of His, the sulfhydryl of Cys, the amine of Lys, or the phenol of Tyr), then the amino acid always found in that sequence position likely has an important role in the enzyme's mechanism(s) of catalysis or its overall structure. Positions that have only a few different amino acids harboring chemically similar side chains (such as Leu, Ile, or Val) in the aligned sequences are considered to be somewhat conserved and may be involved in important interactions with substrates, cofactors, or other parts of the enzyme. Sequence positions that vary more widely, meaning that representative sequences have different types of amino acids with chemically dissimilar side chains (such as Glu, Lys, and Leu) in that position, are not conserved. It can be concluded that the common overall structure of the set of aligned proteins is robust to even drastic changes to the amino acids in these unconserved positions. Going beyond individual sites, stretches of sequence with a high density of highly or somewhat conserved positions are likely to have structural or functional importance. Often, conservation will be high in stretches of sequence that contribute to the active site. Sequence alignments can thus provide guidance as to regions of the enzyme that are likely to tolerate substitutions without loss of function and other regions in which any substitution, or particular classes of substitutions, will likely disrupt the enzyme's structure and cause a loss of activity. *See e.g.*, Ex. 1023 (Elkins), 5190 (Fig. 5); Ex. 1042 (Schleinitz), 5360 (Fig. 2).

2. Principles for Designing Enzyme Variants

52. By 2004-2005, it was common for skilled artisans to create enzyme variants with desired functional properties. A fundamental paradigm in the art was this established link between protein sequence and function: an enzyme's amino acid sequence determines its three-dimensional fold (structure), and its structure determines its biochemical function (activity). Therefore, persons skilled in the art generally analyzed an enzyme's amino acid sequence by comparing it to other known, similar sequences. Ex. 2025 (Minshull), 203 ("sequences of orthologous proteins are thus a potentially rich source of information regarding sequence changes that are acceptable without drastically disrupting protein function").

53. When making enzyme variants, a person skilled in the art in 2004-2005 would not have randomly picked any of the 20 possible amino acids for a given residue, as Dr. Hausinger suggests, but would have instead relied on known principles for generating enzyme variants. Ex. 1054, ¶ 79.

54. To guide these substitutions, known matrices such as PAM or BLOSUM, which were commonly used to score alignments of protein sequences, would have provided guidance as to which amino acid replacements have been accepted by the processes of natural selection. *See, e.g.*, Ex. 2057 (Dayhoff), 345; Ex. 2041 (Müller), 761. These matrices were developed from sets of high-confidence alignments of many homologous proteins by assessing the frequencies of all possible

amino acid substitutions. Ex. 2025 (Minshull), 205. They show which changes are favored or disfavored, providing guidance to skilled researchers on how to modify enzymes without losing activity. Ex. 2025 (Minshull), 205-206.

55. As noted above, skilled artisans performed sequence alignments with related proteins to identify highly conserved residues and segments that likely delineate important structural or functional elements. Comparing amino acid sequences with low identity (~30%) was particularly helpful here because residues found to be conserved even at a relatively high level of divergence among aligned sequences are likely important to structure, function, or both, whereas sets of sequences with higher identity will have more coincidental identities at positions that may not have important roles.

56. A person skilled in the art in 2004-2005 would have had access to computational tools that considered information provided by the substitution scoring matrices and sequence alignments to predict which substitutions in a given enzyme would be tolerated and which would cause loss of function. For example, SIFT was one available program that predicted whether an amino acid substitution would affect protein function by considering the position at which the change occurred and the type of amino acid change. Ex. 2044 (Ng), 3812.

57. Skilled artisans also used directed evolution techniques (often combined with rational design) that mimic natural evolution to produce variants. Ex.

2038 (Chica), 378 (targeting "multiple, specific residues to mutate on the basis of prior structural or functional knowledge [to] create 'smart' libraries"). Such approaches enhanced directed evolution by incorporating strategies based on known sequence-activity relationships to efficiently generate enzyme variants. *See id.*

58. In summary, a skilled person would have followed several fundamental scientific principles of protein engineering in 2004-2005 when designing enzyme variants:

1. Maintain high sequence identities in view of the known sequence-function correlations for enzymes. Skilled artisans understood that high levels of sequence identity between enzymes are generally correlated with the same or similar function. By 2004-2005, studies consistently confirmed that the minimum threshold for confidently predicting enzyme function (e.g., typically 60-70%) was well below the 85% threshold recited in the '555 patent claims. Ex. 2019 (Tian); Ex. 2028 (Rost 2002); Ex. 2025 (Minshull).

2. Maintain the active site motif. The active site residues are those in the enzyme's interior pocket that allow the enzyme to push and pull on the substrates to catalyze a chemical reaction. A skilled person would be careful to preserve the active site motif residues. Ex. 2025 (Minshull), 204-05 ("changes at key active site residues or other highly conserved residues are generally deleterious").

3. Retain other non-motif residues that are highly conserved across related enzymes. This is particularly true for enzymes with low sequence identities (pairwise identities of ~30%) where "changes at well-conserved positions tend to be deleterious." Ex. 2044 (Ng), 3812; Ex. 2025 (Minshull), 204-05.

4. Maintain charge and steric bulk. Skilled artisans understood not to introduce mutations that would change the residue's charge or significantly change steric bulk. *See* Ex. 1042 (Schleinitz), 5360 Fig. 2 (recognizing amino acid residue changes across αKG-dependent dioxygenases, including changes that maintain residue charge such as V to I). For instance, Müller et al. tested the effect of various mutations, including one that resulted in a bulkier side chain and presented steric hindrance that interfered with the enzyme's reactive conformation. Ex. 1035 (Müller 2006), 1360. Skilled artisans would therefore avoid mutations that add too much side chain bulk. Skilled artisans, recognizing that changing an amino acid's charge or polarity to the opposite charge would likely affect enzyme function, would have also avoided these types of substitutions. Ex. 2044 (Ng), 3812.

59. Once enzyme variants had been obtained by following these principles, a skilled artisan would have understood that the genes encoding the enzymes could be used for plant transformation for expression in plants.

C. α-Ketoglutarate-Dependent Dioxygenases

1. Reactions Catalyzed

60. Before 2004-2005, researchers identified bacteria that could degrade 2,4-D and other phenoxyacetate herbicides. This recognition led to the identification of the gene encoding the TfdA enzyme from the bacterium *Alcaligenes eutrophus* JMP134 in 1997.[2] Ex. 1044 (Streber). TfdA was initially mischaracterized as a monooxygenase. Ex. 2001 (Fukumori), 24311. In 1993, Fukumori et al. identified the reaction by which TfdA degrades 2,4-D and established that TfdA belongs to the family of α-ketoglutarate-dependent ("αKG-dependent") dioxygenase enzymes. Ex. 2001 (Fukumori), 24311.

61. To degrade 2,4-D, an αKG-dependent dioxygenase requires the presence of Fe(II) as the metallocenter (metal cofactor), as well as oxygen and α-ketoglutarate (αKG) as co-substrates. Ex. 2001 (Fukumori), 24311. When TfdA acts on 2,4-D as its "primary substrate," the enzymatically catalyzed oxidation reaction produces carbon dioxide, succinate, 2,4-DCP (a phenol), and glyoxylate, as

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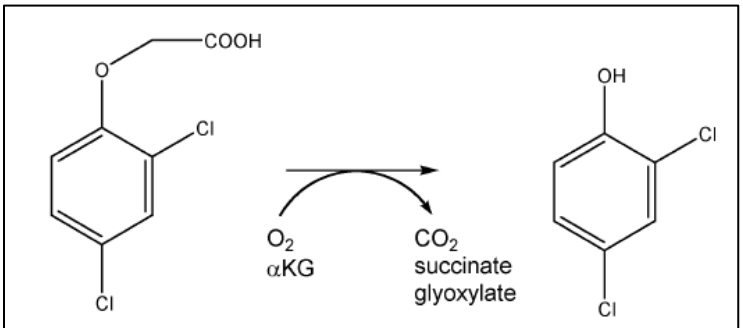
[2] *A. eutrophus* JMP134 was later renamed *Ralstonia eutropha* JMP134, and then renamed *Cupriavidus necator* JMP134. The *tfdA* gene is encoded by the pJP4 plasmid in this strain. In this declaration, I will refer to the canonical JMP134 TfdA enzyme as "TfdA," unless otherwise noted.

shown in the scheme below. Ex. 1025 (Hausinger), 21, 40 (scheme 33). 2,4-D, similar to FOP herbicides, were cleaved at the ether bonds to produce phenol compounds.



SCHEME 33.

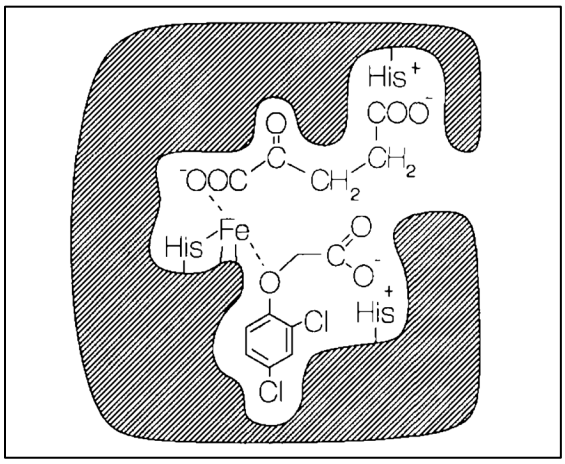
62. The αKG-dependent dioxygenase family is large. Ex. 1025 (Hausinger), 21. In addition to enzymes like TfdA that are involved in detoxification, the family includes enzymes involved in plant biosynthetic pathways, antibiotic biosynthesis, and lipid metabolism. Ex. 1025 (Hausinger), 21, 29-38.

63. Given the size of this family, members of the αKG-dependent dioxygenase family were generally classified into one of three groups of related enzymes. Ex. 1027 (Hogan), 12400-401. TauD, a family member that is closely related to TfdA, was classified in the same group (Group II) as TfdA. Ex. 1027 (Hogan), 12401. TauD nevertheless has a different function than TfdA; it catalyzes the conversion of αKG, O_2 and taurine to CO_2, succinate, aminoacetaldehyde, and sulfite, which is used as a source of sulfur. Ex. 1023 (Elkins), 5185.

2. The αKG Motif and How It Was Determined

64. αKG-dependent dioxygenases are generally defined by their consensus motif, which is a simple way to identify their active sites. By 2004-2005, skilled artisans recognized that enzymes in the αKG-dependent dioxygenase family often varied greatly in the exact numbering of the motif residues and the number of amino acids in the spacers separating each pair of consecutive motif residues, reflecting the diversity of reactions catalyzed by these enzymes. Ex. 1023 (Elkins), 5185.

65. In 1993, researchers depicted an αKG-dependent dioxygenase active site (i.e., of TfdA) that accommodates the required Fe(II) ion and αKG co-substrate to catalyze the degradation of 2,4-D:



Ex. 2001, 24316 (Fig. 11). Through further investigations, researchers identified the particular active-site residues that anchor the Fe(II) metallocenter (e.g., the two His residues and the Asp residue in the AAD-1 motif) and the α-ketoglutarate co-substrate (e.g., the Thr and Arg residues in the AAD-1 motif) in the active site. The

substrates and Fe(II) are anchored in a configuration that orchestrates the oxidation reaction to cleave the primary substrate's ether bond. The motif residues that interact with Fe(II) and αKG are interspersed in different parts of the sequence, and the protein fold brings them together in the active site to allow them to orchestrate the reaction and create the pocket for binding of the primary substrate such as 2,4-D. In this way, the spacer sequences separating the motif residues that anchor Fe(II) and αKG set the contours for the active site and help define the specificity of the enzyme for its primary substrate(s).

66. The specific motif for each αKG-dependent dioxygenase family member was recognized as a distinctive characteristic of the enzyme, with those particular motif residues and their spacings routinely identified in literature and published sequence alignments, including by Dr. Hausinger:

TABLE 1

Metal ligands and αKG-binding residues of selected Fe(II)/αKG-dependent hydroxylases and related enzymes

Enzyme	Ligand 1	Ligand 2	Spacing	Ligand 3	Spacing	αKG C-5 stabilization
Human prolyl 4-hydroxylase	His 412	Asp 414	68	His 483	9	Lys 493
Human lysyl hydroxylase	His 638	Asp 640	49	His 690	9	Arg 700
Human HIFα-specific prolyl hydroxylase-1	His 135	Asp 137	58	His 196	8	Arg 205
Human FIH	His 199	Asp 201	77	His 279	NA	Lys 214, Tyr 145, Thr 196
Escherichia coli AlkB	His 131	Asp 133	53	His 187	16	Arg 204
Streptomyces clavuligerus CAS	His 144	Glu 146	132	His 279	13	Arg 293, Thr 172
Streptomyces clavuligerus DAOCS	His 183	Asp 185	57	His 243	14	Arg 258, Ser 260
Erwinia carotovora CarC	His 101	Asp 103	147	His 251	11	Arg 263, Thr 130, Arg 253
Penicillium decumbens EpoA	His 131	Asp 133	40	His 174	10	Arg 185
Dactylosporium sp. proline 4-hydroxylase	His 109	Asp 111	103	His 215	10	Arg 226
Streptomyces sp. strain TH1 proline 3-hydroxylase	His 107	Asp 109	48	His 158	9	Arg 168, Ser 170, His 135
Petunia hydrida flavanone 3β-hydroxylase	His 220	Asp 222	55	His 278	9	Arg 288, Ser 290
Citrus unshiu flavonol synthase	His 221	Asp 223	53	His 277	9	Arg 287, Ser 289
Arabidopsis thaliana ANS	His 232	Asp 234	53	His 288	9	Arg 298, Tyr 217, Asn 215
Cucurbita maxima gibberellin 7-oxidase	His 182	Asp 184	53	His 238	9	Arg 248, Ser 250
Cucurbita maxima gibberellin 20-oxidase	His 243	Asp 245	53	His 299	9	Arg 309, Ser 311
Caranthus roseus desacetoxyvindoline-4-hydroxylase	His 308	Asp 310	55	His 366	9	Arg 376, Ser 378
Hyoscyamus niger hyoscyamine 6β-hydroxylase	His 217	Asp 219	54	His 274	9	Arg 284, Ser 286
Human trimethyllysine hydroxylase	His 201	Glu 203	146	His 350	13	Arg 364
Pseudomonas sp. AK1 β-butyrobetaine hydroxylase	His 209	Asp 211	138	His 350	11	Arg 362
Human β-butyrobetaine hydroxylase	His 202	Asp 204	142	His 347	12	Arg 360
Human phytanoyl-CoA hydroxylase	His 175	Asp 177	86	His 264	10	Arg 275
Salmonella typhimurium LpxO	His 155	Asp 157	44	His 202	9	Arg 212
Escherichia coli TauD	His 99	Asp 101	153	His 255	10	Arg 266, Thr 126
Pseudomonas putida S-313 AtsK	His 108	Asp 110	153	His 264	10	Arg 275, Thr 135
Pseudomonas stutzeri WM88 hypophosphite/αKG dioxygenase	His 116	Asp 118	87	His 206	10	Arg 217
Ralstonia eutropha TfdA	His 114	Asp 116	146	His 263	10	Arg 274
Pseudomonas syringae pv. *Phaseolicola* ethylene-forming enzyme	His 189	Asp 191	76	His 268	8	Arg 277
Escherichia coli Gab protein	His 160	Asp 162	129	His 292	12	Arg 305
Aspergillus (Emericella) nidulans IPNS	His 214	Asp 216	53	His 270	NA	NA
Tomato ACC oxidase	His 177	Asp 179	54	His 234	9	Arg 244

NA, not applicable.

Ex. 1025 (Hausinger 2004), 22 (annotated) (identifying the motif residues and specific "Spacing" between them for each enzyme); Ex. 1023 (Elkins 2002), 5190 (Fig. 5 identifying the motif residues with variable spacing due to alignment gaps,

using black triangles); Ex. 1042 (Schleinitz 2004), 5360 (Fig. 2, similarly identifying the motif residues using black shading).

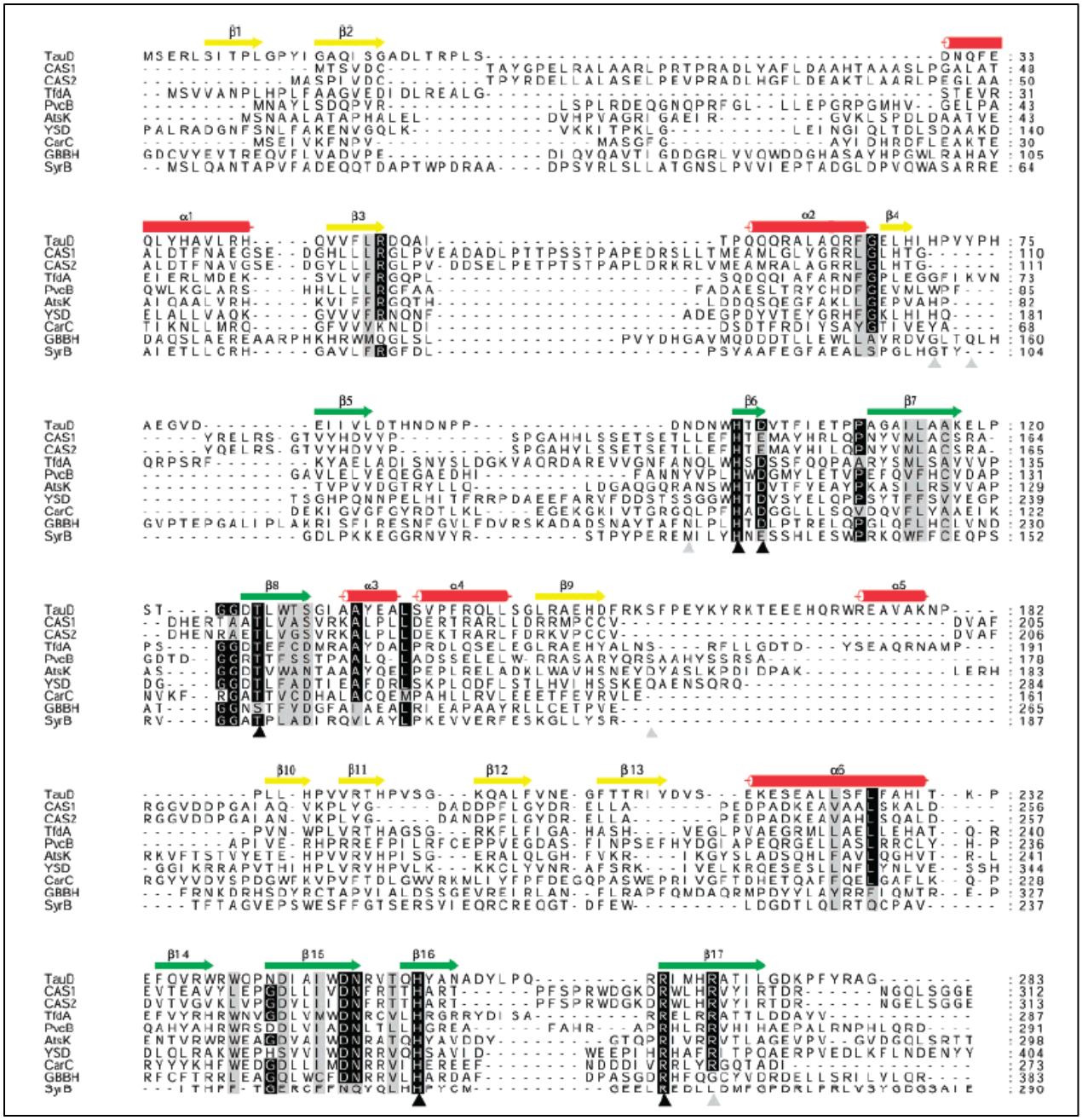
67. In the early 2000s, Hogan et al. identified the metal binding residues in the active site motif of TfdA. Ex. 1027 (Hogan 2000), 12400. They defined the motif of Group II enzymes as **H*X*(D/E)*X*$_{138–207}$H*X*$_{10–13}$R**, which is distinct from the motif of αKG-dependent enzymes in other groups. Ex. 1027 (Hogan 2000), 12401. The letters in the motif represent amino acids: "H" is His (histidine), "D/E" means that this residue must be either Asp (aspartate) or Glu (glutamate), and "R" is Arg (arginine). "X" can be any amino acid, and the numeric subscripts refer to the number of amino acids in the spacers. Hogan et al. also performed an alignment with TauD, revealing that it shared the common H*X*(D/E)*X*$_{138-207}$H*X*$_{10-13}$R motif. Ex. 1027 (Hogan 2000), 12401, 12407 (Fig. 8). Other enzymes in the family, such as the Group I enzymes, were found to share a common H*X*DX$_{50-70}$H*X*$_{10}$(R/K)*X*S motif and to act on different substrates. Ex. 1027 (Hogan), 12401.

68. In 2002, Elkins et al. reported the crystal structure of TauD and used it to predict the structure of TfdA. Ex. 1023 (Elkins). Two of the motif residues, T$_{126}$ and R$_{266}$, were found to interact with αKG through a hydrogen bond and a salt bridge, respectively. Ex. 1023 (Elkins), 5188. The authors further elaborated that the Group II αKG-dependent dioxygenases share the consensus motif **H*X*(D/E)*X*$_{23-26}$(T/S)*X*$_{114–183}$H*X*$_{10–13}$R**. Ex. 1023 (Elkins), 5185. Elkins further explained that since "TfdA is

particularly well characterized," it was "worth modeling to extend our knowledge of the αKG-dioxygenase superfamily" and its predicted overall fold was expected to extend to other "closely related proteins (~30% sequence identity)." Ex. 1023 (Elkins), 5189.

69. Moreover, a 2004 review article by Hausinger described several αKG-dependent enzymes known to have this $HX(D/E)X_{23\text{-}26}(T/S)X_{114\text{-}183}HX_{10\text{-}13}R$ motif, including TfdA, CarC or carbapenem synthase, shown in Scheme 11 and TauD or taurine/αKG dioxygenase, shown in Scheme 29. Ex. 1025 (Hausinger), 22, 31, 39. In the motif of CarC, H_{101}, D_{103}, and H_{251} ligate Fe(II), while T_{130} and R_{263} bind to αKG. *Id.* at 30. In the motif of TauD, H_{99}, D_{101}, and H_{255} ligate Fe(II), while T_{126} and R_{266} bind to αKG. *Id.* at 39. While CarC and TauD share the common family motif and the five conserved residues (H, D, T, H, and R), they each have unique spacing within the motif and catalyze different reactions. CarC facilitates carbapenem stereoinversion and desaturation with a motif of $HXDX_{26}TX_{120}HX_{11}R$, whereas TauD hydroxylates taurine with a motif of $HXDX_{24}TX_{128}HX_{10}R$. *See id.* at 30, 39; *see also* Ex. 1023 (Elkins), 5185. This correlation is consistent with a skilled artisan's understanding in 2004-2005 that enzymes with different functions usually have different active site motifs (i.e., the spacing between conserved motif residues), in addition to having rather low sequence identity.

70. The analysis of Elkins et al. in 2002 suggested that TfdA and other Group II αKG-dependent dioxygenases (generally having ~30% or higher sequence identity) share similar primary and secondary structures. Ex. 1023 (Elkins), 5189. For instance, Figure 2 of Elkins et al. (replicated below) shows the conserved residues and secondary structures in Group II αKG-dependent dioxygenases, including shared α-helices and β-strands of the jellyroll motif, among others. The TauD secondary structure is colored red for helix, green for β-strands of the jellyroll motif, and yellow for other β-strands. Ex. 1023 (Elkins), 5188 (Fig. 2). The motif residues are marked with black triangles at the bottom of the sequence alignment.



Ex. 1023 (Elkins), 5190 (Fig. 5).

3. RdpA

71. In addition to TfdA, researchers had discovered other enzymes produced by soil bacteria that degrade phenoxy auxin herbicides such as 2,4-D.

These enzymes included RdpA enzymes having identical sequences from the bacteria *Delftia acidovorans* MC1 and *Sphingomonas herbicidovorans* MH. Ex. 1044 (Streber); Ex. 1035 (Müller 2006).

72. In 2004, using a sequence alignment, researchers compared the amino acid sequences of four αKG-dependent dioxygenases, RdpA, SdpA, TfdA, and TauD. Ex. 1042 (Schleinitz), 5360 (Fig. 2).

```
RdpA MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFH 50
TauD ------------MSLTIQPISPALGAIVSGIDLGAPLDDTGQRAIEQALL 38
TfdA ------------MSVVANPLHPLFAAGVEDIDLREALGSTEVREIERLMD 38
SdpA ----------MQTTLQITPTGATLGATVTGVHLAT-LDDAGFAALHAAWL 39

RdpA TYQVIYFPGQAITNEQHIAFSRRFGPVDPVPLLKSIEGYPEVQMIRREAN 100
TauD EHQVLFFRDQSLEPRSQARFAARFGDLHIHPIYPSVPEQPEVIVLDTAVT 88
TfdA EKSVLVFRGQPLSQDQQIAFARNFGPLEGGFIKVNQRPSRFKYAELADIS 88
SdpA QHALLIFPGQHLSNDQQITFAKRFGAIERIGGGDIVAISNVKADGTVRQH 89

RdpA ESGRV---------------IGDDWHTDSTFLDAPPAAVVMRAIDVPEHG 135
TauD DVR-----------------DNAIWHTDVTFLETPALGAVLAAKQLPPYG 121
TfdA NVSLDGKVAQRDAREVVGNFANQLWHSDSSFQQPAARYSMLSAVVVPPSG 138
SdpA SPAEWDDMMKVIVG-------NMAWHADSTYMPVMAQGAVFSAEVVPAVG 132

RdpA GDTGFLSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRRFSNT 185
TauD GDTLWASSTAAYEALSAPLRRLLDGLTATHDIGKSFP---RERFGVTEAD 168
TfdA GDTEFCDMRAAYDALPRDLQSELEGLRAEHYALNSRF---------LLGD 179
SdpA GRTCFADMRAAYDALDEATRALVHQRSARHSLVYSQS----KLGHVQQAG 178

RdpA SVKVMDVDAGDRETVHPLVVTHPGSGRKGLYVNQVYCQRIEGMTDAESKP 235
TauD LARLEEARLKNPPRSHPVVRTHPVTGRKGLFVSDGFTTRINELEPAESDA 218
TfdA TDYSEAQRNAMPPVNWPLVRTHAGSGRKFLFIG-AHASHVEGLPVAEGRM 228
SdpA SAYIGYGMDTTATPLRPLVKVHPETGRPSLLIG-RHAHAIPGMDAAESER 227

RdpA LLQFLYEHATRFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTR 285
TauD LLKFLFAHATRPEFTVRWRWQENDVAFWDNRVTQHYAVDDYRPQRRVMHR 268
TfdA LLAELLEHATQREFVYRHRWNVGDLVMWDNRCVLHRGRRYDISARRELRR 278
SdpA FLEGLVDWACQAPRVHAHQWAAGDVVVWDNRCLLHRAEPWDFKLPRVMWH 277

RdpA TTVGGVRPAR-----                                    295
TauD ATILGDKPF------                                    277
TfdA ATTLDDAVV------                                    287
SdpA SRLAGRPETEGAALV                                    292
```

FIG. 2. Sequence alignment of RdpA and SdpA with other group II αKG-dependent dioxygenases. TfdA, *R. eutropha* 2,4-D/αKG dioxygenase (accession no. A27082); TauD, *P. aeruginosa* taurine/αKG dioxygenase (accession no. NP_252624). Conserved Fe(II)- and αKG-binding residues are shaded in black; other conserved regions are shaded in gray. Black arrows indicate 2,4-D binding residues in TfdA; gray arrows indicate residues undergoing posttranslational hydroxylation in TauD or TfdA. Amino acid positions numbers are shown to the right of each line. The alignment was created with CLUSTAL W.

Ex. 1042 (Schleinitz), 5360 (Fig. 2).

73. In each of the four sequences, Schleinitz 2004 highlighted the conserved motif residues with black shading (H, D, T, H, and R), explicitly marked because of their functional importance. Ex. 1042 (Schleinitz), 5360 (Fig. 2) ("Conserved Fe(II)- and αKG-binding residues are shaded in black"). Schleinitz also numbered the residues on the right-hand side of the alignment, as was often done in the field. *See* Ex. 1023 (Elkins), 5190 (Fig. 5). Schleinitz thus clearly discloses the numbered positions of each motif residue in the RdpA sequence: first residue (H) at position 111; second residue (D) at position 113; third residue (T) at position 138; fourth residue (H) at position 270; and fifth residue (R) at position 281. Ex. 1042 (Schleinitz), 5360 (Fig. 2). The residue spacing between the active-site residues is numbered in the same manner, with those spacer residues located at positions 112, 114-137, 139-269, and 271-280. *Id.*

74. These conserved motif residues were reported to be "functionally important" and "functionally essential." Ex. 1042 (Schleinitz), 5357, 5362. Further, a skilled artisan would have noticed the gaps in the alignment, understanding that these gaps indicate that the spacing between the conserved motif residues is different for each enzyme. *See id*. RdpA is the only enzyme in the alignment having no gaps, illustrating a difference in its active site motif as compared to other enzyme family members. *See id*. For example, the lack of gaps illustrates that RdpA has the longest spacer sequence (residues 139-269) between the conserved T and H. *See id.* The

different motifs of these different αKG enzymes would have been unsurprising to one skilled in the art in 2004-2005, given their functional differences.

VI. Level of Skill in the Art

75. Regarding aspects of the ’555 patent relating to enzymology, particularly αKG-dependent dioxygenases (e.g., RdpA/AAD-1) and variants thereof, a person skilled in the art would have generally possessed a Ph.D. in molecular biology, biotechnology, or biochemistry, and 3-5 years of experience in the development of enzymes and enzyme variants as well as familiarity with αKG-dependent dioxygenases. This need for a background in enzymology and familiarity with αKG-dependent dioxygenases is reflected in the scientific literature from 2004-2005. *E.g.*, Ex. 1023 (Elkins); Ex. 2019 (Tian); Ex. 2028 (Rost 2002); Ex. 2025 (Minshull); Ex. 1042 (Schleinitz).

VII. The ’555 Patent

A. The Claims

76. The ’555 patent contains two independent claims and thirty-two dependent claims. These claims include transgenic plants and plant cells comprising a recombinant polynucleotide encoding AAD-1 proteins having required structural features.

77. Claim 1 of the patent reads as follows:

1. A transgenic plant cell comprising a recombinant polynucleotide that encodes an AAD-1 protein that exhibits

aryloxyalkanoate dioxygenase activity wherein said activity enzymatically degrades a phenoxy auxin herbicide and an (R)-aryloxyphenoxypropionate herbicide, further wherein said AAD-1 protein comprises:

i) *an amino acid sequence having at least 85% sequence identity with SEQ ID NO: 9*; and

ii) an AAD-1 motif having the general formula of:

$HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$, wherein

X_{112} represents a single amino acid at position 112, relative to the sequence of SEQ ID NO: 9;

$(X)_{114\text{-}137}$ represents a sequence of 24 amino acids;

$(X)_{139\text{-}269}$ represents a sequence of 131 amino acids; and

$(X)_{271\text{-}280}$ represents a sequence of 10 amino acids.

Ex. 1001 ('555 Pat.), 165:2-19.

78. The "AAD-1 motif" relates to the active site discussed above for RdpA (AAD-1). It specifies five conserved (fixed) amino acids, as well as the specific spacing between these conserved residues.

79. Independent claim 34 recites features similar to those of independent claim 1 but further requires the AAD-1 motif to have 90% sequence identity with SEQ ID NO: 9 over the stretch of its sequence that corresponds to positions 111 to 281 of SEQ ID NO: 9. Ex. 1001 ('555 Pat.), 166:64-167:17.

80. The dependent claims recite further features, including:

- An AAD-1 motif with the general formula $HX_{112}D(X)_{114-137}T(X)_{139-269}H(X)_{271-280}R(X)_{282-284}R$ (claim 2);
- Plant cells comprising a recombinant polynucleotide that encodes an AAD-1 protein with at least 90% amino acid sequence identity to SEQ ID NO: 9 (claim 3);
- Monocot or dicot plant cells (claims 4, 5);
- A transgenic plant comprising a plurality of the plant cells of claim 1, wherein the polynucleotide expression renders the plant tolerant to an aryloxyalkanoate herbicide (claim 6);
- Tolerance to specific herbicide classes or herbicides (claims 8-10);
- A transgenic plant further comprising a second herbicide resistance gene (claims 11, 12);
- Methods of controlling at least one weed in a field, the field containing at least one plant of claim 6 and methods requiring use of particular herbicides (claims 13-23, 27-29, 31) or particular herbicide resistance genes (claims 24-26); and
- Seeds, progeny, etc., comprising a plurality of the plant cells of claim 1 (claims 30, 32, 33).

B. The Specification

81. The ’555 patent describes transgenic plants expressing AAD-1 αKG-dependent dioxygenase enzymes that can degrade two different classes of herbicides: phenoxy auxins (e.g., 2,4-D) and aryloxyphenoxypropionates (e.g., quizalofop or haloxyfop). Ex. 1001 (’555 Pat.), 4:32-65, 15:40-47. The ’555 patent discloses the amino acid sequence of wild-type RdpA/AAD-1 as SEQ ID NO: 9 and the sequences of two AAD-1 variants as SEQ ID NOs. 10 and 11. Ex. 1001 (’555 Pat.), 6:56-63.

82. The ’555 patent describes the inventors’ identification of an αKG-dependent dioxygenase (RdpA) with unique function based on searching for homologues that have a relatively low sequence identity to TfdA. Ex. 1001 (’555 Pat.), 3:15-32, 44:14-35. Consistent with the function-prediction principles described above (Section V.A.3), the patent explains that “highly identical” sequences among αKG-dependent dioxygenases generally had the same function. Ex. 1001 (’555 Pat.), 44:20-27. The inventors thus sought sequences having lower than 50% sequence identity to TfdA (i.e., extending into the twilight zone). Ex. 1001 (’555 Pat.), 44:26-35. Example 1 reports that the inventors identified RdpA (AAD-1), which had only 28% sequence identity to TfdA. Ex. 1001 (’555 Pat.), 44:37-44. In subsequent studies *in vitro* and *in planta*, the inventors demonstrated the unique functional capability of AAD-1 enzymes with their dual herbicide-

degrading activity. *E.g.*, Ex. 1001 ('555 Pat.), 68:36-80:10 (identifying degradation of phenoxy auxin and aryloxyphenoxypropionate herbicides).

83. The '555 patent discloses that "[p]referred enzymes and genes for use according to the subject invention are referred to herein as AAD-1 (AryloxyAlkanoate Dioxygenase) genes and proteins." Ex. 1001, 18:15-18, 18:32-36, 19:52-54, 20:8-12, 20:30-32. Beyond disclosing the AAD-1 v1, v2, and v3 sequences, the '555 patent specification provides structural guidance for variant enzymes "having the same or similar activity of the exemplified proteins." Ex. 1001, 37:41-42. Consistent with recognized principles of sequence-function relationships in 2004-2005, the '555 patent discloses that such variants will typically have at least 60% sequence identity, including 85% sequence identity. *See* Ex. 1001, 37:37-47; *see also* Ex. 2019 (Tian), 866 (describing high degrees of function conservation between enzymes at greater than 60% sequence identity). Indeed, as Example 1 discloses, enzymes having 85% or higher sequence identity are "highly identical" and "expected to retain similar properties." Ex. 1001, 44:26-29.

84. The patent further explains, for instance, that αKG-dependent dioxygenases, which include RdpA, contain "a '$HX(D/E)X_{23\text{-}26}(T/S)X_{114\text{-}183}HX_{10\text{-}13}R$' motif which comprises the active site." Ex. 1001, 18:1-9; Ex. 1027 (Hogan), 14200; Ex. 1023 (Elkins), 5185. There is only one such set of motif residues in SEQ ID NOs: 9, 10, and 11, which share the same motif residues and spacing. Ex. 1001,

17:66-18:9. This specific motif, with its distinctive HXD triad and the different spacings between the remaining motif residues as compared to other αKG-dependent dioxygenases, was readily apparent to skilled artisans. *See* Ex. 1042 (Schleinitz), Figure 2. The specification further explains that the histidines in the motif coordinate the Fe^{+2} ion in the active site that is essential for catalytic activity. Ex. 1001, 18:1-9.

85. The '555 patent discloses that "[v]arious properties and three-dimensional features of the protein can also be changed without adversely affecting the activity/functionality of the protein." Ex. 1001 ('555 Pat.), 38:6-8. The specification also describes that "[c]onservative amino acid substitutions can be tolerated/made to not adversely affect the activity and/or three-dimensional configuration of the molecule." Ex. 1001 ('555 Pat.), 38:8-11. By 2004-2005, skilled artisans recognized that substituting similarly charged or sized amino acids represented an effective strategy for making variants having the same or improved activity as the reference enzyme.

86. The patent further describes AAD-1 enzymes and their ability to impart resistance to several herbicides through twenty-six examples, extensive data, and twenty-two figures.

87. For instance, Example 5 discloses the preparation and analysis of a "plant-optimized" (v3) AAD-1 gene that encodes the AAD-1 protein. Ex. 1001 ('555 Pat.), 55:36-65:16. Example 5 also provides the design of a soybean-codon-biased

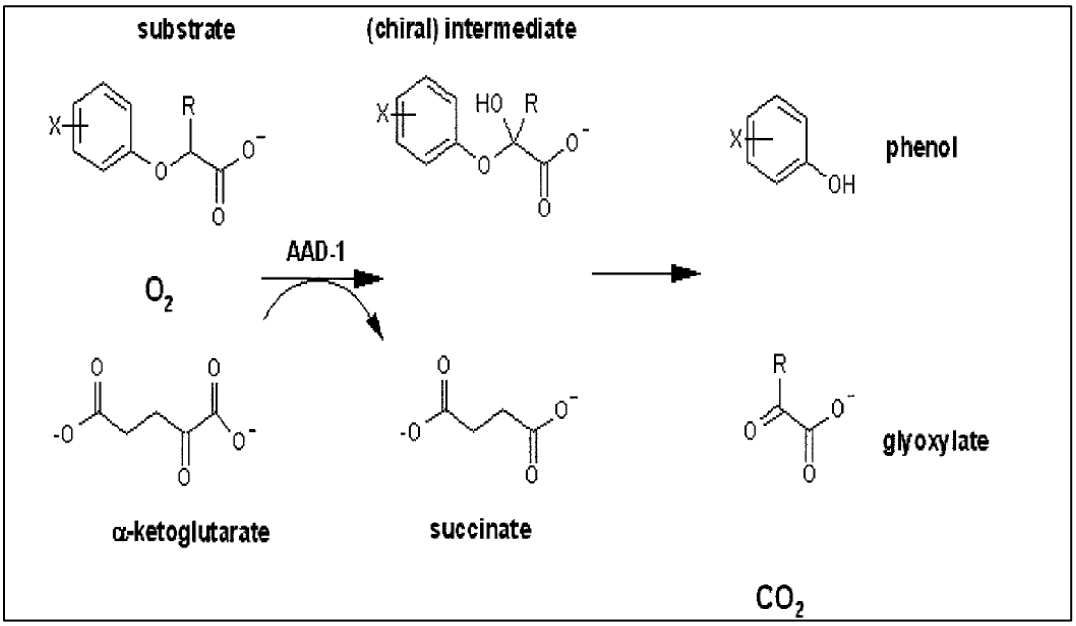
DNA sequence encoding an EPSPS enzyme having mutations that confer glyphosate tolerance. Ex. 1001 ('555 Pat.), 55:36-65:16. Tables 12 and 13 of the patent disclose codon comparisons for making AAD-1 variants that are optimally expressed in plants.

88. Example 5 also discloses the preparation of expression and transformation vectors for AAD-1 (v1, v2, and v3). Ex. 1001 ('555 Pat.), 59:25-60:67; 64:1-65:16 (Table 14). Examples 3 and 4 report the determination of enzymatic activity of AAD-1 using high-throughput *in vitro* assays measuring phenol production by colorimetric and spectrophotometric assays. Ex. 1001 ('555 Pat.), 51:50-55:34.The specification describes a 96-well microplate format for performing enzyme assays measuring degradation activity on 2,4-D and FOPs using incubation times of 15 minutes. Ex. 1001 ('555 Pat.), 51:40-56. The '555 patent reports *in vitro* testing data showing that "AAD-1 (v1) has activity on graminicidal aryloxyphenoxypropionate herbicides as well as phenoxy auxins." Ex. 1001 ('555 Pat.), 52:15-60, Figs. 6A and 6B.

89. In Example 2, the '555 patent reports the results of an investigation into AAD-1 enzymes' mechanism of action on FOP herbicides. The results showed that degradation of both 2,4-D and FOP herbicides resulted in cleaved phenol products, indicating a similar mechanism of action on these substrates. *Id.*, 49:30-60. Further bioassay testing, including HPLC, colorimetric assay, and bioassay results,

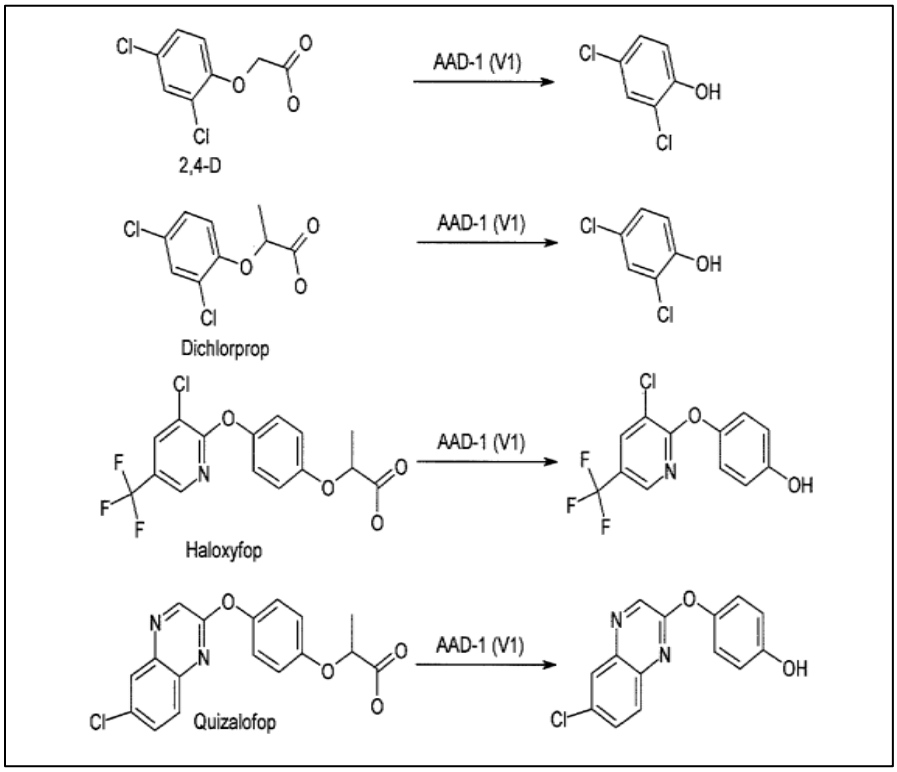
confirmed this activity on FOP herbicides. *Id.*, 50:35-67. The patent provides "the first reported observation for any members of this class of enzyme to be active on herbicides outside the phenoxy auxins." *Id.*, 50:61-64.

90. In Figure 1, the patent illustrates the role of AAD-1 in cleaving the ether bond of either phenoxy auxin or AOPP herbicides to produce a phenol product:



Id., Fig. 1 (excerpt), 5:16-17 ("FIG. 1 shows a general scheme for dioxygenase cleavage of phenoxy auxin or AOPP herbicides."). The patent also discloses that AAD-1 requires "α-ketoglutarate as a co-substrate and ferrous iron to bind in the active site." *Id.*, 49:30-41. The same bioassays, under the same conditions, were used to test degradation of 2,4-D and FOPs by AAD-1, including HPLC results and a colorimetric assay monitoring production of a phenol product. *Id.*, 50:35-42 ("ACCase inhibitors, haloxyfop and diclofop, were then tested using the same

conditions as with 2,4-D"). In Figure 4, AAD-1 degradation of 2,4-D and FOP herbicides is illustrated, showing the phenol product resulting from each herbicide:



Id., Fig. 4, 5:22-23 ("FIG. 4 shows anticipated phenols produced from representative herbicides catalyzed by AAD-1."). The patent discloses that AAD-1 is sufficiently versatile to degrade both 2,4-D and FOPs, despite them having different modes of action as herbicides. *See id.*, 50:64-67. Before this time, RdpA/AAD-1 degradation of FOPs had never been described, let alone by a mechanism similar to that of 2,4-D degradation.

91. Example 6 describes the transformation of *Arabidopsis* plants with an AAD-1 gene. Ex. 1001 ('555 Pat.), 65:17-80:10. Seeds from those transformed plants were collected, and first-generation plants grown from that seed (i.e., T_1 seed)

were tested for dual herbicide-resistance activity. Ex. 1001 ('555 Pat.), 67:7-80:10. A skilled person reading Example 6 would understand that AAD-1 transformed plants had "robust resistance" to the phenoxy auxins and FOPs but not to the pyridyloxyacetic auxins. Ex. 1001 ('555 Pat.), 77:28-33 (Table 20). A skilled artisan would also understand that the AAD-1 (v3) gene imparted to *Arabidopsis* plants herbicide resistance to 2,4-D. Ex. 1001 ('555 Pat.), 78:46-61 (Fig. 9A, 9B).

92. Example 17 reports comparative testing of AAD-1-transformed *Arabidopsis* against *Arabidopsis* transformed to express a different αKG-dependent dioxygenase enzyme (AAD-2). Ex. 1001 ('555 Pat.), 111:9-118:13. The amino acid sequence of AAD-2 is substantially different from that of AAD-1 (only 29% identity). *See* Ex. 1001 ('555 Pat.), 111:19-25. In this study, the inventors found that AAD-1-transformed plants exhibited more robust 2,4-D resistance than AAD-2-transformed plants. Ex. 1001 ('555 Pat.), 115:58-118:13.

93. Examples 7, 8, 12-14, 19, 20, 24, and 26 report investigations of monocot and dicot plants transformed with an AAD-1 gene, including soybeans, tobacco, cotton, corn, rice, and canola. In Example 14, transformed cotton plants were tested for tolerance to the herbicide 2,4-D. Ex. 1001 ('555 Pat.), 106:41-110:20. The '555 patent discloses that "the AAD-1 (v3) gene in cotton provides substantial tolerance to 2,4-D." Ex. 1001 ('555 Pat.), 110:18-20. The '555 patent also describes the use of combinations of 2,4-D and AOPP herbicides with AAD-1-transformed

dicot crops such as soybean. Ex. 1001 ('555 Pat.), 119:8-120:13 ("One skilled in the art of weed control will now recognize that use of various commercial phenoxy auxin herbicides alone or in combination with any commercial AOPP herbicide is enabled by AAD-1 (v3) transformation."); Ex. 1001 ('555 Pat.), 90:18-22 ("These results indicate that most AAD-1 (v3) transformed event lines displayed a high level of resistance to quizalofop and 2,4-D at rates that were lethal or caused severe epinastic malformations to non-transformed corn hybrids."). A person skilled in the art, reading these examples, would also understand that the '555 patent describes successful testing of AAD-1-transformed rice to both classes of herbicides. Ex. 1001 ('555 Pat.), 125:9-128:28.

94. Taken together, these examples disclose to a person skilled in the art that (1) plants comprising an AAD-1 gene can degrade phenoxy auxin herbicides (such as 2,4-D) and aryloxyphenoxypropionic acid herbicides (such as quizalofop and haloxyfop); and (2) a plant expressing an αKG-dioxygenase enzyme with at least 85% sequence similarity to AAD-1 and the claimed AAD-1 motif was reasonably expected to exhibit the dual herbicide degrading function. *See, e.g.*, Ex. 1001 ('555 Pat.), 4:32-4:56.

VIII. The '555 Patent and Its Priority Applications Adequately Describe AAD-1 Enzymes

95. As referenced above in Section IV, I have been informed that the '555 patent claims are entitled to priority of the 2004-2005 filing dates if the specification

in 2004-2005 provides written description of the claimed invention. I understand that the written description requirement can be satisfied either by disclosure of common structural features correlated to function or by disclosure of species (here, enzymes) representative of the AAD-1 enzymes of the ’555 patent claims. In my opinion, the specification discloses both.

A. The ’555 Patent Discloses Common AAD-1 Structural Features Correlated to the Dual Herbicide-Degrading Function

96. The ’555 patent discloses two structural features (“at least 85% sequence identity” and an “AAD-1 motif”) common to the AAD-1 enzymes of the claims. These structural features in the ’555 patent are correlated to their function of degrading the two specified classes of herbicides, phenoxy auxins (e.g., 2,4-D) and *R*-aryloxyphenoxypropionates (e.g., quizalofop or haloxyfop), and thus provide sufficient written description for making and using AAD-1 enzyme variants. Accordingly, in my opinion, the specification provides sufficient written description support for the claimed set of AAD-1 enzymes.

1. The Common Structural Feature of at Least 85% Sequence Identity with SEQ ID NO: 9 Is Correlated to the Dual Herbicide-Degrading Function

97. The first common structure of the ’555 patent claims is the requirement of “at least 85% sequence identity to SEQ ID NO: 9.” Ex. 1001 (’555 Pat.), 165:8-9. SEQ ID NO: 9 is the amino acid sequence of the RdpA enzyme from *Sphingobium herbicidovorans* disclosed in the ’555 patent as degrading phenoxy auxin and

aryloxyphenoxypropionate herbicides. Ex. 1001 ('555 Pat.), 4:32-65; Ex. 1004, ¶¶ [0059]-[0061]; Ex. 1005-1008, 1021, ¶¶ [0018]-[0020]. A codon-optimized AAD-1 (v3) gene was reported to express an AAD-1 enzyme that degraded phenoxy auxin and aryloxyphenoxypropionate herbicides in many plant species. Ex. 1001 ('555 Pat.), Examples 6, 7, 8, 12, 21, 22.

98. The specification discloses that the proteins and genes "of the subject invention are referred to herein as AAD-1 proteins and genes." Ex. 1001 ('555 Pat.), 19:52-54; Ex. 1004-1005, ¶ [0069]; Ex. 1006-1008, 1021, ¶ [0095]. In my opinion, a skilled artisan would have generally expected enzymes having at least 85% sequence identity to SEQ ID NO: 9 and the AAD-1 motif to be AAD-1 enzymes possessing the disclosed dual herbicide-degrading activity. My opinion is supported by the '555 patent's disclosures as well as the skilled person's background knowledge of enzymology in 2004-2005.

99. The '555 patent discloses that proteins "having the same or similar activity of the exemplified proteins," such as SEQ ID NO: 9, will typically be within a sequence identity of "at least 60%." Ex. 1001 ('555 Pat.), 37:37-47; Ex. 1004, ¶ [00116]; Ex. 1005-1008, 1021, ¶ [00142]. It also specifies higher sequence identities, including 85% identity and above. Ex. 1001 ('555 Pat.), 37:44-55; Ex. 1004, ¶ [00116]; Ex. 1005-1008, 1021, ¶ [00142]. Among αKG-dependent dioxygenases, the specification further discloses that enzymes having 85-98%

identity are "highly identical" and that "sequences with high homology would be expected to retain similar properties." Ex. 1001 ('555 Pat.), 44:17-35; Ex. 1005-1008, 1021, ¶ [00171]. A skilled artisan would have understood that such high levels of sequence identity between enzymes are correlated with having the same or similar function.

100. The '555 patent's disclosures are consistent with the background knowledge of a skilled artisan. By 2004-2005, several research groups had independently analyzed many thousands of protein sequences to determine threshold levels of sequence identity at which common function can be reliably predicted, as well as the levels of sequence identity where enzyme function begins to differ. Ex. 2019 (Tian), 863; Ex. 2024 (Wilson), 233; Ex. 2026 (Devos), 98; Ex. 2027 (Todd), 1113; Ex. 2028 (Rost 2002), 595. These studies demonstrated that at sequence identities of about 60-70% and higher, the function of a known enzyme can be reliably transferred to (i.e., predicted for) an enzyme whose function has not yet been experimentally confirmed. At higher sequence identities, the correlation was even stronger. In other words, there was a recognized trend that higher sequence identities more strongly correlate to conservation of enzyme function (and vice versa).

101. Tian et al., for instance, explained in 2003:

> [B]ecause the percentage of sequence identity is ***simple, quick and widely accepted*** by the biologist, ***several groups have examined the extent to which pairwise sequence identity [to]***

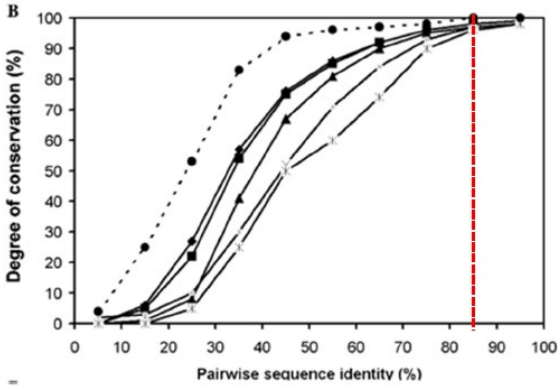
> ***function similarity can be inferred***. Devos, Wilson, and Todd [i.e., three separate studies] conducted an all-against-all pairwise enzyme sequence comparison, and concluded that ***when the sequence identity is above 40%, above 90% accuracy can be achieved*** when transferring the full four digits [of an] EC number.

Ex. 2019 (Tian), 872. Tian et al. reviewed and built upon prior studies by these other groups (Devos (Ex. 2026), Wilson (Ex. 2024), and Todd (Ex. 2027)). Ex. 2019 (Tian), 864. Tian's analysis reported that "above 60% sequence identity is needed to have above 90% accuracy" for transferring all four digits of an EC number. Ex. 2019 (Tian), 876; *see* Section V.A.3, above.

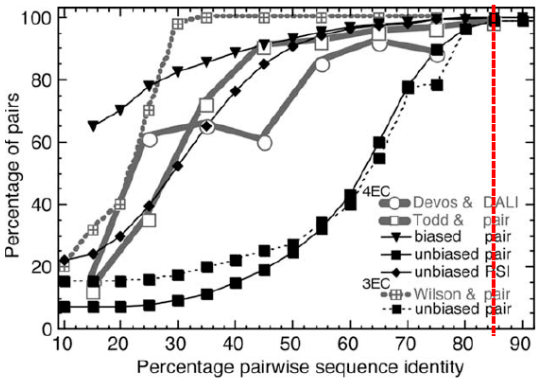
102. Another study by Rost in 2002 (also considered by Tian et al.) found that at least 70% sequence identity was required to predict enzyme function. Ex. 2028 (Rost 2002); *see* Section V.A.3, above. Rost reported estimates for sequence conservation of enzyme function that were "more cautious" than previous studies but provided "important benchmarks for everyday sequence analysis." Ex. 2028 (Rost 2002), 595; Ex. 2024, 245 ("The sequence and function similarity thresholds potentially establish minimum thresholds of sequence similarity for reliable function prediction.").

103. These studies collectively confirmed that the minimum threshold for reliably predicting enzyme function was well below the 85% sequence identity

threshold recited in the ’555 patent claims. This conclusion is illustrated by the figures below from Rost and Tian, to which I have added the red line annotation to indicate 85% sequence identity. Both figures show that, at 85% sequence identity, the degree of enzyme function conservation (shown on the y-axis) is well above 90% and nearing 100%. Thus, skilled artisans would have considered the “at least 85% sequence identity” feature of the ’555 patent claims as conservatively defining a narrow sequence space in which proteins would be expected to have the same function as SEQ ID NO: 9.



Ex. 2019 (Tian), 866 (Fig. 1(B)).



Ex. 2028 (Rost 2002), 604 (Fig. 5).

104. Dr. Hausinger asserts that a skilled artisan would not have been able to predict, based on the information in the patent, whether any given amino acid sequence possessing the AAD-1 motif and having at least 85% sequence identity would have had the claimed activity. Ex. 1054, ¶¶ 87-91, 72-75. I disagree. His statement is contradicted by the studies discussed above, which confirmed the reliability with which skilled artisans in 2004-2005 predicted protein function based

on sequence. Moreover, a skilled artisan would have appreciated that the sequence-based conservation of enzyme function was related to the strong conservation of enzyme structure at high levels of sequence identity. *See, e.g.*, Ex. 2028 (Rost 2002), 596 ("In general, transfer of structure is almost guaranteed to be correct above the twilight zone, e.g., all known pairs of proteins with more than 33 residues in 100 identical have similar structure."); Ex. 2021 (Rost 1999), 85 ("Sequence alignments unambiguously distinguish between protein pairs of similar and non-similar structure when the pairwise sequence identity is high (> 40% for long alignments)."); Ex. 2026 (Devos), 105 ("Structural similarity is conserved even at very low levels of sequence similarity.").

105. Indeed, skilled artisans understood in 2004-2005 that an enzyme's tertiary structure is at least as conserved as (if not more conserved than) an amino acid sequence based on the structure-and-function principles discussed above. Because enzyme structure can be quite forgiving to amino acid substitutions, skilled artisans generally based protein-function predictions on sequence identity rather than other structural comparisons. Ex. 2024 (Wilson), 238 ("Structure is always more highly conserved than sequence, so usually a given amount of structural similarity is not as significant as a corresponding amount of sequence similarity."); Ex. 2028 (Rost 2002), 604 ("When two sequences are very similar we can safely infer that they have similar structures."). Accordingly, persons skilled in the art in

2004-2005 would have predicted that, at or above the claimed threshold of at least 85% identity to SEQ ID NO: 9, the tertiary structure of a given enzyme would be sufficient to maintain the function of degrading phenoxy auxin and aryloxyphenoxypropionate herbicides.

106. Dr. Hausinger also states that 85% sequence identity to SEQ ID NO: 9 cutoff is an "arbitrary" number that allows for a significant range of sequence variation, such that the genus of proteins with the claimed (*R*)-enantioselective activity could not be defined. Ex. 1054, ¶¶ 91, 71. I disagree. A skilled artisan would have understood that at least 85% sequence identity defines a narrow sequence space well within established ranges for conserving enzyme function. Ex. 2019 (Tian). Contrary to Dr. Hausinger's opinion, as discussed above, Tian, Rost, and other researchers demonstrated that, for enzymes having 85% or more sequence identity, a skilled artisan would have expected conserved function. Ex. 2019 (Tian), Fig. 1(b); Ex. 2028 (Rost 2002), Fig. 5. Dr. Hausinger does not appear to have considered any of this foundational literature in the field for predicting function based on sequence identity. Identifying other types of enzymes having different activities within 85% sequence identity would have been considered highly unusual.

107. Moreover, by 2004-2005, persons skilled in the art used data from known sequence-activity relationships not just to predict the function of newly discovered sequences, but also to make new protein variants. For example, Minshull

et al. reported that the sequences of "orthologous" proteins—i.e., related proteins (e.g., from a different organism but having a similar function) and typically having lower sequence similarity—are a "potentially rich source of information regarding sequence changes that are acceptable without drastically disrupting protein function." Ex. 2025 (Minshull), 202-204 (citing the creation of new enzymes as a main reason to "predict an enzyme's function from its sequence"). Thus, skilled artisans were using sequence comparisons in 2004-2005 to provide structural guidance about which mutations would likely be tolerated or not tolerated.

108. Further, contrary to Dr. Hausinger's opinions, it was clear from the specification that the disclosed sequence identity ranges (including at least 85% sequence identity) and protein variants correspond to AAD-1 (SEQ ID NO: 9), which is a "specifically exemplified" sequence throughout the specification. Ex. 1001, 37:36-55; Ex. 1004, ¶ [00116]; Ex. 1005-1008, 1021, ¶ [00142]; Ex. 1054, ¶¶ 71, 91. A skilled artisan would have readily understood that AAD-1 proteins and genes were part of the invention, as AAD-1 was mentioned hundreds of times in the patent and was the focus of the examples. Ex. 1001, 19:52-59 ("In light of the novel activity, proteins and genes of the subject invention are referred to herein as AAD-1 proteins and genes"); Ex. 1004-1005, ¶ [0069]; Ex. 1006-1008, 1021, ¶ [0095]. The specification also explicitly lists sequence identities at which equivalent proteins to AAD-1 can be expected, including 85% or higher sequence identity. Ex. 1001,

37:36-55; 165:6-10; Ex. 1004, ¶ [00116]; Ex. 1005-1008, 1021, ¶ [00142]. It also discloses 85% sequence identity as a percentage at which one would consider two protein sequences to be "highly identical" to one another and therefore expect them to "retain similar properties." Ex. 1001, 44:26-29; Ex. 1005-1008, 1021, ¶ [00171].

109. Accordingly, a sequence identity of 85% or greater to SEQ ID NO: 9, as disclosed in the '555 patent, was recognized to be correlated to enzyme function and used for making enzyme variants that maintain function. Skilled artisans would therefore have expected AAD-1 enzyme variants to retain their dual herbicide-degrading activity when they shared the common structural feature of having at least 85% sequence identity to SEQ ID NO: 9.

2. The Common AAD-1 Active Site Motif Is Correlated to the Dual Herbicide-Degrading Function

110. The second common structure of the '555 patent claims is the AAD-1 motif, $HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$. Ex. 1001 ('555 Pat.), 165:10-19. Thus, in addition to requiring highly similar sequences, the claims further require the AAD-1 enzymes to have the same active site motif as in the dual herbicide-degrading protein sequence of SEQ ID NO: 9. In other words, the claims require a particular active-site motif within the already narrow sequence space ("at least 85% sequence identity with SEQ ID NO: 9"), which further guided skilled artisans to readily recognize the AAD-1 enzymes of the claims.

111. Dr. Hausinger states that "[t]he POSA would not have looked to the generic αKG consensus motif, which describes characteristics that are commonly shared by αKG dioxygenases, for guidance on the characteristics that distinguish RdpA's activity from that of other αKG dioxygenases." Ex. 1054, ¶ 102. His statement misses the point. Skilled artisans understood that each αKG-dependent dioxygenase has the distinctive characteristic of a specific motif, which is strongly correlated to its function. The contours of an individual αKG-dependent dioxygenase's active site would be defined by the spacing and residues of its specific motif. A skilled artisan thus would not look to the generic consensus motif, as Dr. Hausinger asserts, instead of the more specific motif within that enzyme's sequence.

112. In the AAD-1 motif, all spacers must have a specified length (1, 24, 131, and 10 amino acids). For the last three spacers, the specified lengths of 24, 131, and 10 amino acids in the AAD-1 motif differ from the spacer lengths found in most other αKG-dependent enzymes. *See, e.g.*, Ex. 1025, 22 (Table 1); Ex. 1023, 5190 (Fig. 5); Ex. 1042, 5360 (Fig. 2). Dr. Hausinger fails to identify any αKG-dependent enzyme that has all four specified spacer lengths, at least 85% identity to SEQ ID NO: 9, and catalyzes a different reaction. I am unaware of any such enzyme. It is irrelevant that other enzymes within the αKG-dependent enzyme family that have one or more than one *different* spacer lengths or less than 85% sequence identity can

have other functions because such enzymes do not fall within the claimed genus of AAD-1 enzymes.

Group II αKG Motif Requirements ($HX(D/E)X_{23\text{-}26}(T/S)X_{114\text{-}183}HX_{10\text{-}13}R$)	**AAD-1 Motif Requirements** ($HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$)
Histidine (H)	Histidine (H)
X (anywhere)	X (anchored relative to position 112 in SEQ ID NO: 9)
Aspartic acid (D) or glutamic acid (E)	Aspartic acid (D)
Anywhere from 23 to 26 variable amino acids ($X_{23\text{-}26}$)	24 variable amino acids (i.e., from positions 114 to 137)
Threonine (T) or serine (S)	Threonine (T)
Anywhere from 114 to 183 variable amino acids ($X_{114\text{-}183}$)	131 variable amino acids (i.e., from positions 139 to 269)
Histidine (H)	Histidine (H)
Anywhere from 10 to 13 variable amino acids ($X_{10\text{-}13}$)	10 variable amino acids (i.e., from positions 271 to 280)
Arginine (R)	Arginine (R)

113. Dr. Hausinger asserts that the specification does not disclose the AAD-1 motif or a genus of enzymes having the AAD-1 motif. Ex. 1054, ¶¶ 63-70, 92-95. I disagree. In 2004-2005, RdpA's primary amino acid sequence alone was sufficient for a skilled artisan to recognize the RdpA (AAD-1) motif. The '555 patent discloses that "[p]referred enzymes and genes for use according to the subject invention are referred to herein as AAD-1 (AryloxyAlkanoate Dioxygenase) genes and proteins." Ex. 1001, 18:15-18, 18:32-36, 19:52-54, 20:8-12, 20:30-32; *See* Ex. 1004-1005, ¶¶ [0060], [0069]; Ex. 1006-1008, 1021 ¶¶ [0086], [0095]. The '555

patent further discloses SEQ ID NO: 9, which provides the enzyme's primary amino acid sequence, including the five motif residues at the numbered locations of the AAD-1 motif. Ex. 1001, 6:56-58, col. 141-144 (SEQ ID NO: 9); Ex. 1004, (page 123, SEQ ID NO: 9), Ex. 1005 (pages 255-57, SEQ ID NO: 9), Ex. 1006 (pages 209-10, SEQ ID NO: 9), Ex. 1007 (pages 205-06, SEQ ID NO: 9), Ex. 1008 (pages 228-229, SEQ ID NO: 9), Ex. 1021 (pages 197-199, SEQ ID NO: 9). The '555 patent also discloses that RdpA is a Group II αKG-dependent dioxygenase and, as such, has five aminos in its acid active-site motif. *See* Ex. 1001, 20:4-12; *See* Ex. 1004, ¶ [0071]; *See* Ex. 1005-1008, ¶ [0097]. As discussed above, the variable motif for Group II αKG-dependent dioxygenases was also disclosed and known in the art, which would have oriented a skilled artisan to the motif residues in the RdpA sequence. *See* Ex. 1023 (Elkins), 5185.

114. Moreover, Dr. Hausinger overlooks that skilled artisans already knew the locations of the motif residues in RdpA (SEQ ID NO: 9) because Schleinitz published that information in 2004, illustrating the exact residues and spacing of the RdpA motif as compared to other Group II αKG-dependent dioxygenases. Ex. 1042 (Schleinitz), 5360 (Fig. 2). For instance, a skilled artisan would have recognized from Schleinitz's sequence alignment that only RdpA has exactly 131 amino acids between T and H.

```
RdpA MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFH 50
TauD -------------MSLTIQPISPALGAIVSGIDLGAPLDDTGQRAIEQALL 38
TfdA -------------MSVVANPLHPLFAAGVEDIDLREALGSTEVREIERLMD 38
SdpA -----------MQTTLQITPTGATLGATVTGVHLAT-LDDAGFAALHAAWL 39

RdpA TYQVIYFPGQAITNEQHIAFSRRFGPVDPVPLLKSIEGYPEVQMIRREAN 100
TauD EHQVLFFRDQSLEPRSQARFAARFGDLHIHPIYPSVPEQPEVIVLDTAVT 88
TfdA EKSVLVFRGQPLSQDQQIAFARNFGPLEGGFIKVNQRPSRFKYAELADIS 88
SdpA QHALLIFPGQHLSNDQQITFAKRFGAIERIGGGDIVAISNVKADGTVRQH 89

RdpA ESGRV----------------IGDDWHTDSTFLDAPPAAVVMRAIDVPEHG 135
TauD DVR-------------------DNAIWHTDVTFLETPALGAVLAAKQLPPYG 121
TfdA NVSLDGKVAQRDAREVVGNFANQLWHSDSSFQQPAARYSMLSAVVVPPSG 138
SdpA SPAEWDDMMKVIVG--------NMAWHADSTYMPVMAQGAVFSAEVVPAVG 132

RdpA GDTGFLSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNRRFSNT 185
TauD GDTLWASSTAAYEALSAPLRRLLDGLTATHDIGKSFP---RERFGVTEAD 168
TfdA GDTEFCDMRAAYDALPRDLQSELEGLRAEHYALNSRF----------LLGD 179
SdpA GRTCFADMRAAYDALDEATRALVHQRSARHSLVYSQS----KLGHVQQAG 178

RdpA SVKVMDVDAGDRETVHPLVVTHPGSGRKGLYVNQVYCQRIEGMTDAESKP 235
TauD LARLEEARLKNPPRSHPVVRTHPVTGRKGLFVSDGFTTRINELEPAESDA 218
TfdA TDYSEAQRNAMPPVNWPLVRTHAGSGRKFLFIG-AHASHVEGLPVAEGRM 228
SdpA SAYIGYGMDTTATPLRPLVKVHPETGRPSLLIG-RHAHAIPGMDAAESER 227

RdpA LLQFLYEHATRFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTR 285
TauD LLKFLFAHATRPEFTVRWRWQENDVAFWDNRVTQHYAVDDYRPQRRVMHR 268
TfdA LLAELLEHATQREFVYRHRWNVGDLVMWDNRCVLHRGRRYDISARRELRR 278
SdpA FLEGLVDWACQAPRVHAHQWAAGDVVVWDNRCLLHRAEPWDFKLPRVMWH 277

RdpA TTVGGVRPAR----- 295
TauD ATILGDKPF------ 277
TfdA ATTLDDAVV------ 287
SdpA SRLAGRPETEGAALV 292
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Ex. 1042 (Schleinitz), 5360 (Fig. 2) (annotated).

115. Moreover, the sequence identities between RdpA and the other Group II αKG-dependent dioxygenases shown in Schleinitz's Figure 2 are low (~30-35%). These differences further illustrate the importance of the AAD-1 motif and having at least 85% sequence identity to SEQ ID NO: 9 for the unique dual herbicide-degrading activity of AAD-1 enzymes disclosed in the '555 patent.

Indeed, Dr. Hausinger does not assert that the other enzymes in the figure exhibit degrading activity against (*R*)-aryloxyphenoxypropionate herbicides.

Enzyme	Active-Site Motif	% Seq Identity to AAD-1
AAD-1	$HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$	100%
TauD	$HX_{98}D(X)_{100\text{-}123}T(X)_{125\text{-}252}H(X)_{254\text{-}263}R$	35%
TfdA	$HX_{115}D(X)_{117\text{-}140}T(X)_{142\text{-}262}H(X)_{264\text{-}273}R$	32%
SdpA	$HX_{109}D(X)_{111\text{-}134}T(X)_{136\text{-}261}H(X)_{263\text{-}272}R$	30%

116. Skilled artisans intuitively understood in 2004-2005 that an enzyme's active-site residues are correlated to its activity. Minshull 2005, for example, confirmed that when making enzyme variants, "changes at key active site residues" were "generally deleterious." Ex. 2025 (Minshull), 204-205. Moreover, Dr. Hausinger and others in the field had often reported the different spacing of the active-site motif residues in αKG-dependent dioxygenases and related enzymes, which results in binding pockets of different sizes and different configurations for catalyzing a diversity of reactions. Ex. 1025 (Hausinger 2004), 21-22 (Table 1); Ex. 1023 (Elkins 2002), 5190 (Fig. 5); Ex. 1042 (Schleinitz 2004), 5360 (Fig. 2).

117. Indeed, Schleinitz disclosed that RdpA can degrade *R*-dichlorprop and identified the motif residues in RdpA as "functionally important" and "functionally essential," as a skilled artisan would have expected. Ex. 1042 (Schleinitz 2004), 5357 (confirming that "the functionally important amino acid sequence motif" is present in RdpA), 5362 ("functionally important"). In 2004, it was not known that

RdpA (AAD-1) could degrade FOP herbicides. The ’555 patent’s inventors, however, investigated AAD-1’s activity on other substrates and confirmed that AAD-1 can degrade FOP herbicides by the same mechanism and in the same active site as for phenoxy auxins, producing a characteristic phenol product from the FOP substrate degradation. Ex. 1001 (’555 Pat.), 49:30-50:67. A skilled artisan thus would have readily understood that the AAD-1 motif is essential to the claimed dual-herbicide degrading activity.

118. Accordingly, as the ’555 patent discloses, and as a skilled artisan would have further recognized from the prior literature in the field, the conserved residues and exact spacing of the RdpA/AAD-1 motif differ from the active-site motif of other αKG-dependent dioxygenases and correlate with the claimed dual herbicide-degrading function.

3. The Patent Provides Sufficient Structural Information to Distinguish AAD-1 from Other αKG Family Members

119. Dr. Hausinger argues that, without crystal structure data, there was insufficient information “to distinguish the amino acid sequences of ‘AAD-1 proteins’ with the claimed activity from those of the other αKG dioxygenases.” Ex. 1054, ¶¶ 105, 104-108. Further, Dr. Hausinger asserts that Chekan et al. reports the necessity of a crystal structure to recognize “bona fide AADs.” *Id.* I disagree. Contrary to Dr. Hausinger’s assertion, Chekan et al. did not report a blanket requirement of crystal structure to recognize “bona fide” AAD enzymes (notably *not*

AAD-1 enzymes specifically), but rather discussed the utility of structural data in the context of identifying other AAD enzymes in nature having low sequence identity to AAD-1, extending down into the twilight zone. Ex. 1019, 13300 (describing efforts to "locate other candidate AADs" and noting that sequence homology searching is difficult because sequence identities were low, e.g., 28% or 30%); *see also* Ex. 1020, 16 (Figure 10) (aligning the low sequence identity AAD's of AAD-1, AAD-2, and AAD-12). Indeed, the AAD candidates identified by Chekan were sequences from different organisms, *not* an attempt to make or identify AAD-1 variants. Ex. 1019, 13300-13301 (citing Ex. 1020, 17-18 (Table S1)).

120. Chekan thus addressed an issue unrelated to identification of AAD-1 variants within the '555 patent claims, which require high sequence identity (>85%), not low sequence identity extending into the twilight zone. *See supra* Section VIII.A.1. In addition to high sequence identity (>85%), the patent provides significant structural guidance with the AAD-1 motif to distinguish AAD-1 proteins from other enzyme family members. *See supra* Section VIII.A.1 and VIII.A.2. A skilled artisan would have readily identified the motif, as disclosed both by Schleinitz et al. in 2004 and in the patent, and would have easily distinguished it from the motif in other αKG dioxygenases, which do not contain all of the same residues with the same spacing between them (let alone with an overall 85% sequence identity to SEQ ID NO: 9 or higher). *See* Ex. 1042 (Schleinitz), 5360

(Fig. 2); Ex. 1001 ('555 Pat.), 165:10-19. A skilled artisan would also have recognized that proteins at high sequence identity (>85%) would be expected to have the same function as AAD-1. Given that each of these structural features are correlated with AAD-1's unique dual-herbicide degrading function, a crystal structure was not necessary to distinguish or recognize the narrow scope of AAD-1 proteins of claim 1.

121. Additionally, Dr. Hausinger contends that the patent does not provide sufficient structural information to distinguish AAD-1 enzymes from other αKG-depdendent dioxygenases, including AAD-2, and thus to predict the (*R*)-enantioselectivity of an AAD protein. *See* Ex. 1054, ¶ 106. I disagree. Chekan et al. reported that "[b]oth (*R)*-diclofop and (*R*)-cyhalofop **bind at the AAD-1 active site** in a manner similar to that of the phenoxycarboxylic synthetic auxin," similar to the '555 patent's previous disclosure of a common degradation pathway for both phenoxy auxins and FOPs within the same "active site." Ex. 1019 (Chekan), 13302 (emphasis added); Ex. 1001 ('555 Pat.), 49:30-50:67; Ex. 1004-1005, ¶¶ [00163]-[00168]; Ex. 1006-1008, 1021 ¶¶ [00192]-[00198]. Chekan refers to a solvent-exposed tunnel in the crystal structure of AAD-1, but states that those residues have a "lack of significant interactions" with AOPP/FOP herbicides. *Id*., 13302-03. As for AAD-2, Chekan et al. theorize that the ~20 residues in AAD-2 outside of the consensus motif may be involved in its lack of (*R*)-enantioselective activity. *See id*.

at 13303. However, while an interesting theory, it is irrelevant because it is directed to why AAD-2 is *not* (*R*)-specific. Overall, the data in the Chekan et al. study further support the importance of the AAD-1 motif, as disclosed in the original application and patent, for degrading 2,4-D and FOP herbicides.

4. Dr. Hausinger's Reliance on *A. denitrificans* and Saari (1999) Is Irrelevant and Incorrect

122. Dr. Hausinger asserts that "the TfdA-like enzyme from *A. denitrificans*" disclosed in Saari 1999 has "(*R*)-enantioselective activity" and is evidence of enzymes having high sequence identities exhibiting opposite enantiospecificities. Ex. 1054, ¶¶ 109, 91. I disagree with these conclusions.

123. As of 2004-2005, and still today, skilled artisans have found the possibility of different enzyme functions – including opposite enantiospecificities – at high levels of sequence identity interesting and worthy of follow-up research and publication. That is because such enzymes are very unusual in view of the strong correlation between high sequence identity and conserved function I discussed above. Ex. 2019 (Tian), 863. In other words, instances where enzymes have high sequence identities but different functions are rare exceptions that prove the general rule in the field of strong sequence-function correlation among enzymes. One such notable exception was published by Nakajima et al., which reported opposite stereoselectivity between two purified enzymes having high sequence identity

(64%), but still well below the 85% minimum sequence identity disclosed in the ’555 patent. Ex. 2053 (Nakajima), 4876.

124. Citing Nakajima, the authors of Saari 1999 appear to have been intrigued by the possibility that a TfdA-counterpart enzyme in *A. denitrificans* might have high sequence identity to TfdA while exhibiting opposite (*R*)-enantiospecificity. Ex. 1041, 427. This hypothesis has essentially no bearing on RdpA (AAD-1), which was known to have only 30% identity to its (*S*)-enantiospecific counterpart enzyme, SdpA. Ex. 1035 (Müller 2006), 1357. Regardless, Saari 1999 fell well short of demonstrating that any hypothesis of an (*R*)-specific enzyme with high identity to TfdA was correct.

125. Saari 1999 never specifically attributed (*R*)-enantioselective activity to the *A. denitrificans* enzyme sequence that they presented. There was good reason for that caution—the only functional characterization of the (*R*)-enantioselective activity in *A. denitrificans* involved the use of cell extracts, i.e., breaking open cells and examining substrate transformation in the crude mixture that comes out. *See* Ex. 1041, 426-27. Because cell extracts can contain hundreds of different proteins, attributing an activity to a particular enzyme in that mixture without purifying and testing that particular enzyme in isolation is fraught with potential for misattribution. *See* Ex. 1046 at 7163 (FIG. 1). Accordingly, Dr. Hausinger’s hindsight-based attribution of (*R*)-enantiospecific activity to a particular TfdA-like sequence in

A. denitrificans is speculative and, I believe, ultimately erroneous. Ex. 1054, ¶ 109. My opinion is supported by Saari 1999 itself, which used purified enzyme (not crude cell extract) to confirm the stereospecificity of TfdA from strain JMP134. Ex. 1041 (Saari 1999), 425. Nakajima also used purified enzymes, rather than cell extracts, for its analysis of enzyme stereospecificity. *See* Ex. 2053 at 4876.

126. Indeed, Saari 1999 fell well short of connecting the *A. denitrificans* cell-extract data to the functional characteristics of the TfdA-like sequence, which was reportedly identified only by low-stringency hybridization of oligonucleotide probes based on the sequences of $tfdA_{JMP134}$ or $tfdA_{RASC}$ to *A. denitrificans* genomic DNA (i.e., by finding a gene sufficiently similar in nucleotide sequence to $tfdA_{JMP134}$ or $tfdA_{RASC}$ such that its hybridization with the probe was possible), rather than by purification and in vitro demonstration of activity. *See id.* I do not mean to imply that cell extracts are never useful. A skilled person would recognize that cell extracts can be useful when exploring the overall properties of a strain generally. However, the use of cell extracts to assign an unexpected function to one particular endogenous enzyme in a complex crude extract falls far short of the rigor needed to establish such a surprising result.

127. My opinion is supported by the testimony of Dr. Hausinger, who acknowledged the tenuous nature of using cell extracts to assign function to a particular enzyme. Dr. Hausinger stated that, when using crude cell extracts, assays

can "be **compromised** to some extent" and that "having the purified enzyme, you don't have any interfering substances that can give you false readings." Ex. 2016, 42:7-16 (emphasis added). Dr. Hausinger further acknowledged that "other compounds" can interfere with functional assays and inhibit a skilled artisan's ability to assign function. Ex. 2016, 43:2-12. Assaying purified protein, in contrast, permits a skilled artisan to assign a particular, unexpected function to a particular enzyme sequence of interest. Ex. 2016, 42:2-6.

128. My opinion is also supported by further characterizations of *A. denitrificans*, published by Vedler et al. in 2004. Vedler identified the enzyme-encoding sequences in a plasmid of *A. denitrificans*. Ex. 1046, 7164-7165 (Table 1: "Locations and predicted functions of pEST4011 coding sequences"). Consistent with my opinions above regarding the enzyme sequence-function relationships recognized in the field, Vedler predicted the function of the putative *A. denitrificans* protein on the basis of percentage sequence identity to an enzyme of known function. *Id.* Regarding the TfdA-like enzyme previously identified by Saari 1999, Vedler predicted TfdA activity, not RdpA-like activity. *Id.* Indeed, Vedler never mentions any hypothesis or possibility that the TfdA-like enzyme has (*R*)-enantiospecificity (much less any activity on (*R*)-FOPs specifically), and it does not appear that any follow-up research in the field has borne out Dr. Hausinger's speculation.

129. My opinion is still further supported by Schleinitz 2004. Schleinitz noted that while *A. denitrificans* had been "biochemically characterized" as of 2004, there were "no data regarding the genetic background [] yet available." Ex. 1042, 5357. Schleinitz expressed skepticism about the hypothesis that a TfdA-like protein in *A. denitrificans* was responsible for *R*-dichlorprop cleavage, given the lack of *tfdA* expression studies or identified amino acid sequences of the *R*-specific enzyme. *See* Ex. 1042, 5362 ("However, it was not supported by *tfdA* expression studies or amino acid sequences of the *R*-dichlorprop cleaving dioxygenase[]").

130. Accordingly, Dr. Hausinger presented no reliable evidence of sequences having high sequence identity (>85%) and exhibiting opposite stereoselectivity, which would have been highly unusual in the field. A skilled artisan would have instead expected two proteins of high sequence identity (>85%) to exhibit the same function.

B. The AAD-1 Enzymes Disclosed in the Specification Are Representative of the AAD-1 Enzymes of the Claims

131. I have been informed that species are representative when they reflect the structural and functional diversity of the genus in the claims. In my opinion, the genus of AAD-1 enzymes is neither structurally nor functionally diverse, and the AAD-1 enzymes disclosed in the '555 patent are amply representative of the genus.

132. The three exemplary AAD-1 enzymes described in the '555 patent are representative of the claimed AAD-1 enzymes. The claims are narrowly tailored to

AAD-1 enzymes having at least 85% sequence identity to SEQ ID NO: 9 and containing the AAD-1 motif, resulting in minimal variation in the structural features of the AAD-1 enzymes. As detailed above, those structural features are also strongly correlated with the dual herbicide-degrading function, such that a skilled artisan would have expected enzymes that have at least 85% sequence identity to SEQ ID NO: 9 and the conserved active-site motif to exhibit the same function. Accordingly, a skilled artisan would have understood that the disclosed AAD-1 enzymes are sufficiently representative of the structure and function of claimed set of AAD-1 enzymes.

133. A skilled artisan would have considered the claimed set of AAD-1 enzymes as essentially the same enzyme with the same function based on their high sequence identity (85% or more) to SEQ ID NO: 9 and conserved AAD-1 motif. Regarding high sequence identity, the '555 patent accurately describes enzymes having 85% or higher sequence identity as "highly identical" and as "expected to retain similar properties." Ex. 1001 ('555 Pat.), 44:26-29. This was consistent with a skilled artisan's understanding in 2004-2005 that enzyme function and structure are strongly correlated at 85% and higher sequence identity. *See* Section V.A.3 above; Ex. 2019 (Tian), 863 ("several studies suggested that when the sequence identity is above 40%, enzyme function is well-conserved"); Ex. 2028 (Rost 2002),

604 ("When two sequences are very similar we can safely infer that they have similar structures."); Ex. 2021 (Rost 1999).

134. Additionally, as summarized above (Section V.B.2), a skilled artisan would have understood the characteristics of AAD-1 variants within the scope of the claims that would have reasonably been expected to possess the claimed activity. In addition to maintaining high sequence identity (above 85%) and including an AAD-1 motif as the claims require, skilled artisans were amply able to conserve function in enzyme variants by following established principles, including retaining or cautiously modifying highly conserved residues and retaining overall charge. *See* Section V.B.2, above. Following these recognized principles, a skilled artisan would have reasonably expected that AAD-1 enzymes within the claims would exhibit the claimed dual herbicide activity.

135. Dr. Hausinger asserts that the two variant species of AAD-1 disclosed in the patent, AAD-1 v2 and AAD-1 v3, are not representative. *See* Ex. 1054, ¶¶ 81-86. I disagree. The patent discloses that AAD-1 variants should maintain "the same or similar activity" as AAD-1, while maintaining high sequence identity (>85%) and the AAD-1 motif. Ex. 1001 ('555 Pat.), 37:36-55; 18:1-28. A species is representative if it retains the dual herbicide-degrading activity of AAD-1, has at least 85% sequence identity to SEQ ID NO: 9, and maintains the AAD-1 motif. Both AAD-1 v2 and AAD-1 v3 fulfill these requirements and confirm the expectation of

a skilled person that changes can be made while maintaining activity. Other AAD-1 variants satisfying the structural requirements of the claims would still be "highly identical" and expected to "retain similar properties." Ex. 1001, 44:26-29. A skilled artisan would be well-equipped to recognize, with SEQ ID NO: 9 and two motif-retaining, functional variants in hand (v2 and v3), the limited genus of the claims. Amino acid residue changes need not be "adjacent to the five catalytic site residues" to be representative, as Dr. Hausinger asserts. Ex. 1054, ¶ 86.

136. My opinion is further confirmed by a 2019 publication by Bayer scientists, Larue 2019. They disclosed six RdpA variants reported to have "enhanced enzymatic activity on selected herbicides and improved temperature stability[.]" Ex. 1052 (Larue), 2087. Each of these variants, described as "FT enzymes," retained the dual-herbicide degrading activity of RdpA (here, shown through FOPs and 2,4-D). *Id*. Notably, each of these six variants maintained dual-herbicide degrading activity while possessing both >85% sequence identity to AAD-1 and the AAD-1 motif, including the exact spacing between each motif residue. In the table below, I have determined the percent sequence identity of each FT enzyme to RdpA (AAD-1; SEQ ID NO: 9 of the '555 patent). Notably, each FT enzyme is 89-91% identical to RdpA (AAD-1).

Enzyme	% Seq Identity to RdpA
RdpA	100%
FT_C	90.85%
FT_R	89.83%
FT_T	89.83%
FT_Tv7	89.49%
FT_5	90.51%
FT_Tv3	89.15%

Not only does each FT enzyme retain high sequence identity to RdpA, which indicates similar function, but each FT enzyme retains all active site residues (H, D, T, H, and R) and the required spacing between each residue with no alignment gaps:

```
RdpA        MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
FT_5        MHAALSPLSQRFERIAVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
FT_C        MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
FT_R        MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
FT_T        MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
FT_Tv3      MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
FT_Tv7      MHAALTPLTNKYRFIDVQPLTGVLGAEITGVDLREPLDDSTWNEILDAFHTYQVIYFPGQ
            *****:**:::: * ********************************************

RdpA        AITNEQHIAFSRRFGPVDPVPLLKSIEGYPEVQMIRREANESGRVIGDDWHTDSTFLDAP
FT_5        AITNEQHIAFSRRFGPVDPVPLLKSIEGYPEVQMIRREANESGRVIGENWHTDSTFLDAP
FT_C        AITNEQHIAFSRRFGPVDPVPLLKSIEGYPEVQMIRREANESGRVIGDDWHTDSTFLDAP
FT_R        AITNEQHIAFSRRFGPVDPVPLLKSIEGYPEVQMIRREANESSRVIGDDWHSDSTFLDAP
FT_T        AITNEQHIAFSRRFGPVDPVPILKSIEGYPEVQMIRREANESSRFIGDDWHTDSTFLDAP
FT_Tv3      AITNEQHIAFSRRFGPVDPVPILKSIEGYPEVQMIRREANESSRYIGDDWHADSTFLDAP
FT_Tv7      AITNEQHIAFSRRFGPVDPVPILKSIEGYPEVQMIRREANESSRYIGDDWHADSTFLDAP
            **********************:********************.* **::**:********

RdpA        PAAVVMRAIDVPEHGGDTGFLSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNR
FT_5        PAAVVMYAKEIPPYGGDTLFTSMYTAWETLSPTMQATIEGLNVVHSATRVFGSLYQAQNR
FT_C        PAAVVMRAIEVPEYGGDTGFLSMYSAWETLSPTMQATIEGLNVVHSATKVFGSLYQATNW
FT_R        PAAVVMRAIEVPEYGGDTGFLSMYSAWETLSPTMQATIEGLNVVHSATKVFGSLYQATNW
FT_T        PAAVVMRAIEVPEYGGDTGFLSMYSAWETLSPTMQATIEGLNVVHSATKVFGSLYQATNW
FT_Tv3      PAAVVMRAIEVPEYGGDTGFLSMYSAWETLSPTMQATIEGLNVVHSATKVFGSLYQATNW
FT_Tv7      PAAVVMRAIEVPEYGGDTGFLSMYSAWETLSPTMQATIEGLNVVHSATKVFGSLYQATNW
            ****** * ::* :**** * ***:************************:******** *

RdpA        RFSNTSVKVMDVDAGDRETVHPLVVTHPGSGRKGLYVNQVYCQRIEGMTDAESKPLLQFL
FT_5        RFSNTSVKVMDVDAGDRETVHPLVVTHPETGRKGLYVNQVYCQRIEGMSEKESEPLLSFL
FT_C        RFSNTSVKVMDVDAGDRETVHPLVVTHPVTGRRALYCNQVYCQKIQGMTDAESKSLLQFL
FT_R        RFSNTSVKVMDVDAGDRETVHPLVVTHPVTGRRALYCNQVYCQKIQGMTDAESKSLLQFL
FT_T        RFSNTSVKVMDVDAGDRETVHPLVVTHPVTGRRALYCNQVYCQKIQGMTDAESKSLLQFL
FT_Tv3      RFSGTSVKVMDVDAGDRETVHPLVVTHPVTGRRALYCNQVYCQKIQGMTDAESKSLLQFL
FT_Tv7      RFSNTSVKVMDVDAGDRETVHPLVVTHPVTGRRALYCNQVYCQKIQGMTDAESKSLLQFL
            ***.************************ :**:.** ******:*:**:: **:.**.**

RdpA        YEHATRFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTRTTVGGVRPAR
FT_5        FAHATKPEFTCRVRWQEGDVLVWDNLCTQHYAVPDYAGKFRYLTRTTVGGVRPAR
FT_C        YEHATKFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTRTTVAGDKPSR
FT_R        YEHATKFDFTCRVRWKKDQVLVWDNLCTMHRAAPDYAGKFRYLTRTTVAGDKPSR
FT_T        YEHATKFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTRTTVAGDKPSR
FT_Tv3      YEHATQFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTRTTVAGDKPSR
FT_Tv7      YEHATQFDFTCRVRWKKDQVLVWDNLCTMHRAVPDYAGKFRYLTRTTVAGDKPSR
            : ***: :*******::.:********* * *.****************.* :*:*
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Multiple sequence alignment of RdpA and FT enzymes (Ex. 2061 (annotated); Exs. 2012-2014 and 2048-2050)).

137. Accordingly, in my opinion, the disclosed AAD-1 enzymes in the ’555 patent would have been considered representative of the AAD-1 enzymes of the claims, which is further confirmed by the consistent conservation of the AAD-1 motif and high (89-90%) sequence identity in Bayer’s FT (AAD-1) enzymes.

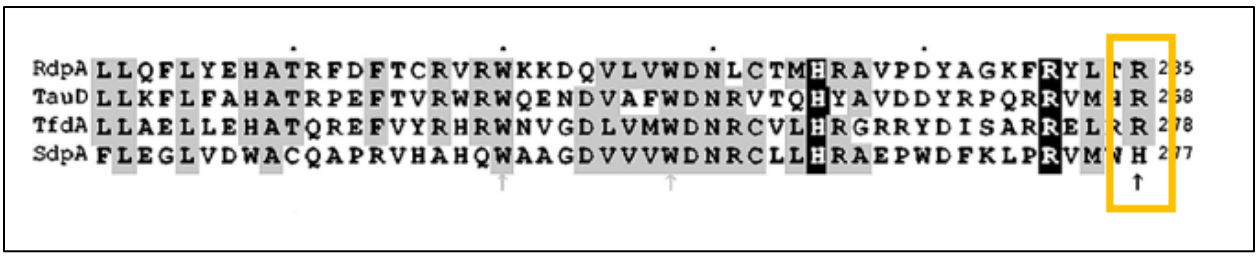
C. Claim 2 of the ’555 Patent Is Adequately Described

138. Claim 2 of the ’555 patent depends from independent claim 1 and includes an additional arginine for the AAD-1 motif, “$HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R(X)_{282\text{-}284}R$, wherein $(X)_{282\text{-}284}$ represents a sequence of three amino acids.” Ex. 1001, 165:23-25. Dr. Hausinger asserts that a skilled artisan would not have understood the patent specification to have described the additional arginine in the motif of claim 2. Ex. 1054, ¶ 96. I disagree.

139. In SEQ ID NO: 9, the specification discloses the additional $(X)_{282\text{-}284}R$ portion of the claim 2 motif, with three residues at positions 282-284 and an arginine at position 285. Moreover, by 2004-2005, skilled artisans were well aware of the role of this additional arginine, which was strongly conserved across other αKG-dependent dioxygenases and known to bind to their primary substrates. As such, it is the type of conserved residue that skilled artisans would have sought to conserve, rather than modify, as I discussed above. *See* Section V.B.2.

140. Indeed, Elkins 2002 disclosed that TauD and TfdA have this conserved arginine residue, which is at position 270 in TauD and position 278 in TfdA. Ex. 1023, 5190 (Fig. 5). Elkins states that “Arg270 is partially conserved across a range of group II dioxygenases (Figure 5), presumably to act as a counterion to the substrate anion,” i.e., to form a ligand to the primary substrate. *Id.*, 5189, 5190 (Fig. 5 marking Arg270 as a binding ligand to TauD’s primary substrate, taurine).

141. Hotopp et al. disclosed in 2002 that for TfdA, the corresponding arginine, Arg278, had "important roles in binding the carboxylate of 2,4-D." Ex. 1029, 9792. Arg278 was reported to be conserved in other αKG-dependent dioxygenases. *Id.* Follow-up work by Schleinitz et al. in 2004 highlighted the same conserved arginine at position 285 in RdpA and its substrate-binding role in TfdA, strongly indicating a similar function for Arg285 in RdpA. Ex. 1042 (Schleinitz), 5360 (Fig. 2).



Ex. 1042 (Schleinitz), Fig. 2 (annotated).

142. Accordingly, a skilled artisan in 2004-2005 would have recognized the functional importance of Arg285 in SEQ ID NO: 9 and understood the specification to describe the additional arginine and its spacing in the claim 2 motif.

IX. The Prior Art Does Not Render the Challenged Claims Obvious as of 2004-2005

143. As referenced above in Section IV, I understand that an obviousness analysis includes evaluating the differences between the claims as a whole and the prior art, including the level of ordinary skill in the art. I have considered whether a person of ordinary skill in the art would have had reasons or motivations to combine

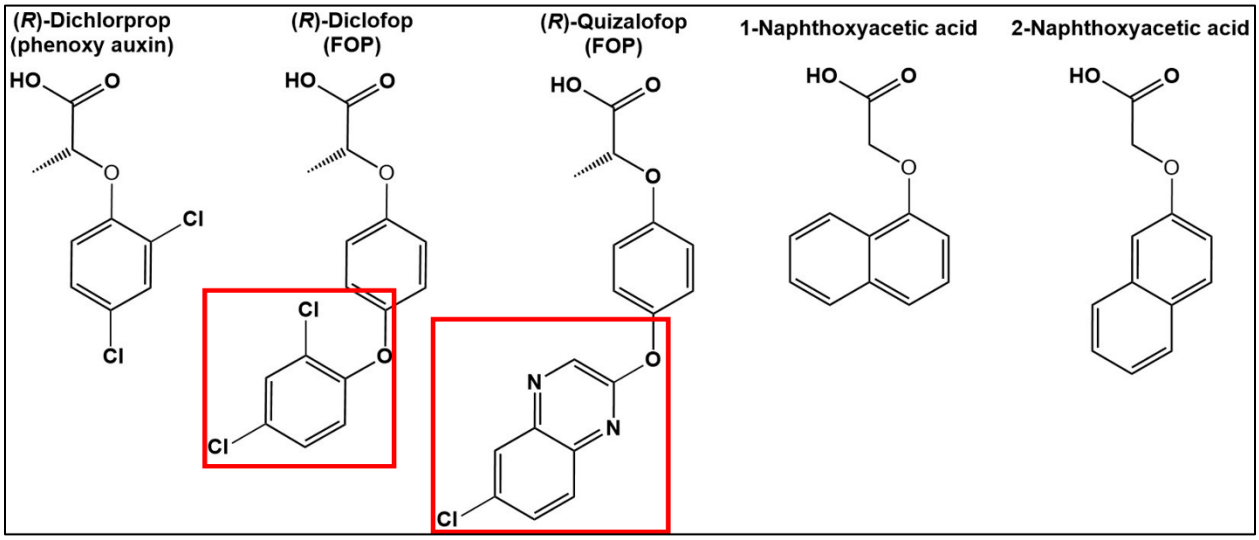
the prior art with a reasonable expectation of successfully achieving the claimed invention. I have also considered whether the claimed invention exhibits unexpected properties or results. In my opinion, the claims differ significantly from the prior art and require a dual herbicide-degrading activity that a skilled artisan would have found surprising and unexpected as of 2004-2005.

A. The Prior Art Provided No Reasonable Expectation of Success of Achieving the Claimed Dual Herbicide-Degrading Function of AAD-1

144. Dr. Hausinger asserts that phenoxy auxins and FOPs are structurally related, which would have led a skilled artisan to expect that RdpA (AAD-1) could degrade both classes of herbicides. Ex. 1054, ¶ 43. I disagree. As of 2004-2005, a person of ordinary skill in the art would not have reasonably expected that RdpA could degrade FOP herbicides, which are a unique class of herbicides compared to phenoxy auxins.

145. From the perspective of one skilled in enzymology, phenoxy auxins and FOPs have notable differences in their structures, particularly if one were considering them as potential substrates for enzymatic degradation. As shown below, FOPs are significantly larger than phenoxy auxins due to the presence of the additional aryl group (enclosed in red). The additional steric bulk in the FOPs would have led a person of skill in the art to doubt that an enzyme that efficiently degrades a phenoxy auxin would also degrade a FOP, just as the ’555 patent explained. Ex.

1001, 3:57-59 ("However, these classes of herbicides are all quite distinct, and no evidence exists in the current literature for common degradation pathways among these chemical classes."); *See* Ex. 1040 (Saari 1998), Figure 8.



As shown above, FOPs are also significantly larger than even the 1- and 2-naphthoxyacetate compounds Dr. Hausinger cites as purportedly "large" and having dual aromatic ring structures. Ex. 1054, ¶ 52.

146. A skilled artisan would have recognized that the structural differences between the phenoxy auxins and FOP classes of herbicides are significant and important. Indeed, these differences are sufficiently profound to give the two classes different biological activities (i.e., different modes of actions in plants). As Dr. Hausinger acknowledges, phenoxy auxins are growth regulators. Ex. 1054, ¶ 39, 43. By contrast, FOPs prevent the formation of fatty acids and lipid production through the inhibition of Acetyl-CoA carboxylase (ACCase). The '555 patent discloses that

phenoxy auxins and FOPs are "distinct" classes of herbicides with unique degradation pathways. Ex. 1001, 3:49-62. Given the significant differences in size and mechanism of action of these two classes of herbicides, a person of ordinary skill in the art in 2004-2005 would not have expected a single enzyme to be capable of degrading members of both classes.

147. As of 2004-2005, the prior art gave no indication that RdpA would degrade FOP herbicides. Dr. Hausinger suggests that the structural modeling of TfdA reported by Elkins et al. in 2002 and RdpA published in 2006 by Müller et al.—which nowhere mention FOPs as possible substrates—would have indicated to a skilled artisan that RdpA would bind both phenoxy auxins and the larger FOP herbicides. *See* Ex. 1054, ¶¶ 52-57. I disagree.

148. While TfdA and RdpA are both αKG-dependent dioxygenases that can degrade 2,4-D, they are different enzymes with different specificities towards other substrates. First, TfdA is stereoselective for the (S)-enantiomers of phenoxy auxins. *See* Ex. 1041 (Saari 1999), 422 ("Here, we demonstrate that purified R. eutropha TfdA specifically degrades the S enantiomer of dichlorprop…"); Ex. 1001, 3:26-42. By contrast, RdpA was disclosed to degrade (*R*)-enantiomers. Second, TfdA has never been reported to degrade FOP herbicides. The '555 patent, in contrast, disclosed (for the first time) that RdpA can degrade FOP herbicides. Ex. 1001, 49:30-50:67. Third, TfdA and RdpA have a relatively low degree of sequence

identity and a different active site motif, with different spacing between the conserved residues. As shown in the table below, TfdA has only 121 residues between T and H, whereas RdpA/AAD-1 has 131 residues.

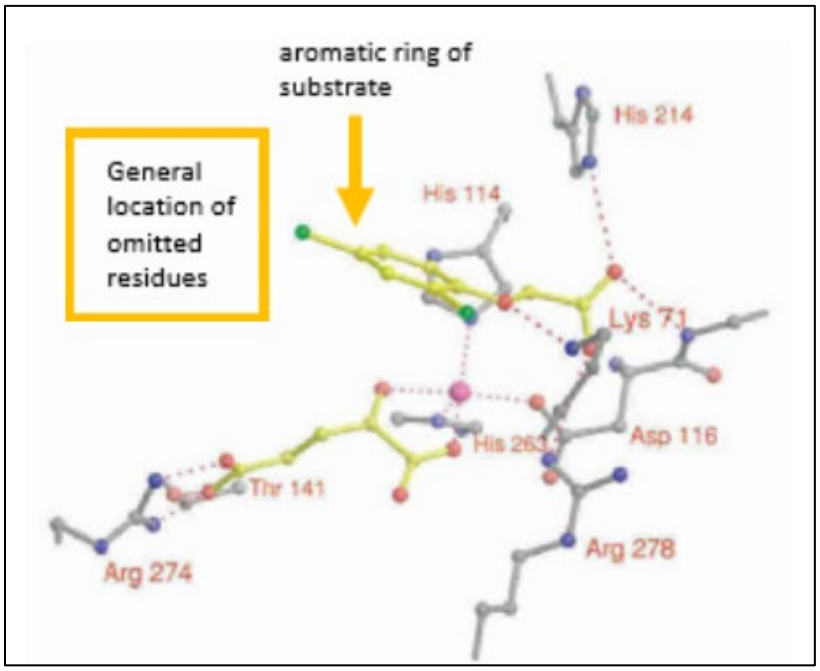
Enzyme	Active-Site Motif	% Seq Identity to AAD-1
AAD-1	$HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$	100%
TfdA	$HX_{115}D(X)_{117\text{-}140}T(X)_{142\text{-}262}H(X)_{264\text{-}273}R$	32%

149. As explained above in Section V.C.2, the spacing within the active site is correlated with an enzyme's function. A skilled artisan would have expected RdpA and TfdA, with their different spacer lengths, to have different active site contours defining different binding pockets for their primary substrates. Accordingly, a person of skill in the art would not have relied upon the TfdA model to formulate expectations regarding the suitability of FOPs as substrates for RdpA/AAD-1. Instead, a person of skill in the art in 2004-2005 would have recognized the important differences between these two enzymes and anticipated differences in their substrate specificities.

150. Elkins et al. also acknowledged that "additional interactions with the substrate are certain to involve residues 86-111, which could not be convincingly modeled," and were thus omitted from Elkins's TfdA model. Ex. 1023 (Elkins), 5189. The absence of information concerning the positions of these 26 residues judged to be "certain" to contact the primary substrate, 2,4-D, would have led a

person of skill in the art to further distrust this model as a basis to predict primary substrate specificity of other enzymes (e.g., RdpA, which has only 32% sequence identity to TfdA). *Id*.

151. Close inspection of the structural model presented in Elkins, et al. would have further led a skilled artisan to expect TfdA *not* to accept a FOP herbicide as a primary substrate. The break in the modeled structure caused by the unmodeled residues 86-111 was located near the 2,4-D substrate in Figure 6 of Elkins, as shown below.

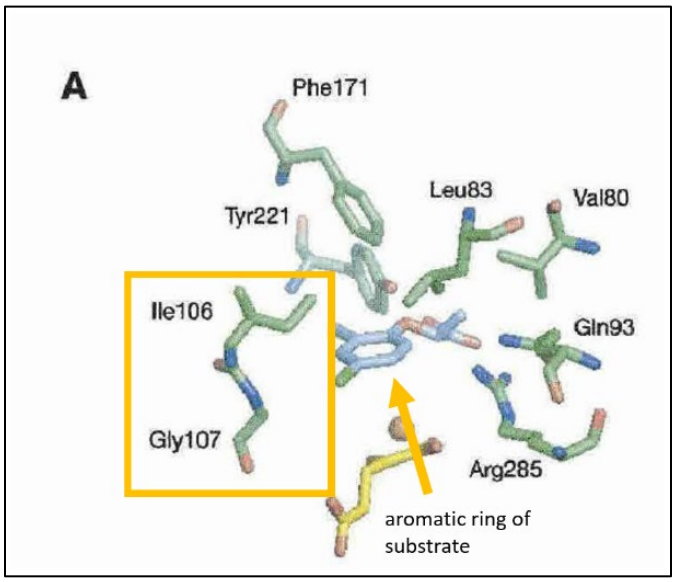


Ex. 1023, 5190 (annotated)

152. In view of the assertion by Elkins, et al. that unmodeled residues 86-111 would certainly contact even the smaller 2,4-D, a skilled artisan would have

expected those same residues to prevent the enzyme from binding the much larger FOP substrates, with their additional aromatic ring structures.

153. Regarding Müller 2006, even entertaining the hypothetical that a skilled person in 2004-2005 could have somehow known about it, the paper provided data that would have suggested to the POSA that substrates much larger than 2,4-D, including the FOPs, would *not* be accommodated in the RdpA/AAD-1 active site and therefore would not be degraded. For example, increasing the size of a side chain in the vicinity of the phenoxy of the modeled mecoprop substrate by substituting G107 with I or N was disclosed to create steric hindrance to the binding of *R*-mecoprop, a phenoxy auxin much smaller than FOPs. *See* Ex. 1035, 1361-62.



Ex. 1035, 1359 (annotated)

A person of skill in the art would have anticipated that significantly increasing the size of the substrate (i.e., creating additional steric bulk in the same region) would

likely cause a significant steric clash, indicating that FOP herbicides would not be substrates.

154. Accordingly, the prior art failed to provide a skilled artisan with a reasonable expectation of success for RdpA to degrade the structurally different (much larger) FOP herbicides in addition to the phenoxy auxins.

B. TfdA Transgenic Plants, Which Failed Commercially, Would Have Provided No Motivation to Create RdpA Transgenic Plants

155. Dr. Hausinger asserts that a skilled artisan would have been motivated to create RdpA-transformed plants. Ex. 1054, ¶¶ 210-218. I disagree. In 2004-2005, a skilled artisan would have recognized that transgenic plants harboring TfdA were commercially unsuccessful and had significant drawbacks. TfdA-containing transgenic tobacco plants had been created, but researchers described these transgenic plants as having been used only as a model. *See* Ex. 1043-1045 (Streber) (demonstrating 2,4-D resistant TfdA tobacco plants), Ex. 2055 (Duke), 165 ("Although none of the researchers were interested in 2,4-D-tolerant tobacco *per se*, tobacco provides a useful model system…"). They reported that the only potential commercial interest was in creating TfdA transgenic cotton plants. Ex. 2055 (Duke), 168 ("While there are several crops that could benefit from being made tolerant to the herbicide 2,4-D, little interest has been shown in any but cotton…Cotton has been the only commercial crop that has been engineered for 2,4-D tolerance").

156. By 1996, however, seven years after Streber's publication, "[n]one of the 2,4-D tolerant plants ha[d] progressed to field trials." *Id.* In 1999, Last and Llewellyn reported that TfdA transgenic plants had restricted plant growth, further limiting their commercial usefulness. *See* Ex. 2052 (Last), 403 ("However, very young T_2 plants, even of the relatively tolerant line H4AtfdA8, were severely retarded in their growth at 2,4-D concentrations as low as 250 mg L^{-1}."). Given this lack of commercial viability, a skilled artisan would not have been motivated to select another αKG-dependent dioxygenase, such as RdpA, to create transgenic plants.

157. Moreover, by 2004-2005, glyphosate had long been used effectively for broad-spectrum weed control for both dicot (broadleaf) and monocot (grassy) weeds. Ex. 1001, 1:30-62, 15:63-64. As the '555 patent states, in the years leading up to 2004-2005 the inventors became interested in glyphosate alternatives due to the emergence of glyphosate-resistance weeds from glyphosate overuse. Ex. 1001, 1:62-2:19. Dr. Hausinger cites no evidence suggesting that a skilled artisan would have turned to (*R*)-phenoxy auxins as a suitable replacement or alternative to glyphosate. To the contrary, a skilled artisan in 2004-2005 would not have turned to *R*-dichlorprop or *R*-mecoprop, as they were relatively limited use herbicides. Dichlorprop was mainly used for brush control of dicot (broadleaf) weeds, while mecoprop was mainly used on cereal crops. *See* Ex. 2056 (Herbicide Handbook),

134, 282. These uses were more limited than 2,4-D (achiral). *See* Ex. 2056 (Herbicide Handbook), 113. Common uses of 2,4-D included dicot (broadleaf) weed control and crop control (wheat, barley, oats, rye, corn, soybeans, etc.), in part because 2,4-D was known to have the benefit of a "relatively short half-life, [with] no significant carryover effects to subsequent crops [] encountered." Ex. 1001, 2:60-3:2; *see* Ex. 2056 (Herbicide Handbook), 113.

158. Accordingly, the prior art as of 2004-2005 would have provided no motivation for a skilled artisan to use another αKG-dependent dioxygenase, much less RdpA specifically. Instead, the claimed invention provides a surprising result that AAD-1 transgenic plants could confer both broadleaf weed control (using 2,4-D) and grassy weed control (using FOP herbicides).

C. AAD-1 Transgenic Plants Conferring Resistance to Both Phenoxy Auxin and FOP Herbicides Was a Surprising and Unexpected Result

159. The dual-herbicide degrading activity of the claimed invention was undoubtedly quite surprising from the perspective of a skilled artisan in 2004-2005. Indeed, that was my reaction when I first learned of AAD-1's unique ability to act on such different substrates and thereby to confer dual-herbicide degrading activity. The '555 patent inventors were the first to report an enzyme that degrades two classes of herbicides, phenoxy auxins and FOPs. *See* Ex. 1001, 4:32-65. As noted previously, the reason for my surprise is that FOPs are significantly larger than

phenoxy auxins. It was highly surprising and unexpected that one αKG-dependent dioxygenase could degrade two herbicides having such different chemical structures.

160. Additionally, as explained above in Section IX.A, a skilled artisan would not have expected one enzyme to degrade two classes of herbicides which exhibited different mechanisms of action. The dual-herbicide degrading ability is significant because FOPs, through inhibiting lipid biosynthesis, are typically used to control monocot (grassy) weeds, while phenoxy auxins (as growth regulators) are typically used to control dicot (broadleaf) weeds, providing broad weed-control through the introduction of a single gene. *See* Ex. 2056 (Herbicide Handbook), 113; Ex. 1001, 21:14-21 ("Still further, the subject invention provides a single gene that can provide resistance to herbicides that control broadleaf weed (auxins) and grass weeds (AOPPs)"). The patented invention allows for efficient replacement of glyphosate-transgenic crops, as glyphosate was previously used to control both broadleaf and grassy weeds. Ex. 1001, 1:30-62, 15:63-64. To my knowledge, this was the first report of an enzyme, expressed in a transgenic plant, with dual-herbicide degrading activity. *See* Ex. 1001, 3:66-4:3 ("Heretofore, there was no expectation or suggestion that a plant with both of these advantageous properties could be produced by the introduction of a single gene"). This was thus a pioneering advance in the agricultural biotechnology industry.

161. Accordingly, the claimed invention provides the unexpected result of dual-herbicide degrading function (phenoxy auxin and FOP herbicides) in plants transformed to express AAD-1 enzymes, i.e., within the highly identical class of enzymes having a conserved active-site motif and at least 85% sequence identity to SEQ ID NO: 9. *See* Sections VIII.A-B.

X. Conclusion

162. For the reasons set forth above, it is my opinion that the subject matter of the ’555 patent claims was adequately described as of 2004-2005, particularly in view of the patent’s extensive disclosure and known enzyme structure-function correlations, including for αKG-dependent dioxygenases such as RdpA (AAD-1), in 2004-2005. It is also my opinion that the ’555 patent claims would not have been obvious over the asserted references in 2004, as Dr. Hausinger asserts.

163. I declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true. I further declare that all statements made herein were made with the knowledge that willful false statements and the like are punishable by fine or imprisonment or both, under Section 1001 of Title 18 of the United States Code.

Date: 4/16/2024

By: J. Martin Bollinger, Jr.

J. Martin Bollinger, Jr., Ph.D.

APPENDIX A

JOSEPH MARTIN BOLLINGER, JR.

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BORN: August 2, 1964

EDUCATION

Postdoctoral — **N.I.H. Postdoctoral Fellow**, January 1993 to May 1995
Harvard Medical School,
Department of Biological Chemistry and Molecular Pharmacology
Advisor: Christopher T. Walsh
"Enzymes in Biosynthesis of the Trypanosomatid Metabolite, Trypanothione."

Graduate — **Doctor of Philosophy, Biochemistry**, 1993
Massachusetts Institute of Technology (1987-1992)
Advisor: JoAnne Stubbe
"On the Chemical Mechanism of Assembly of the Tyrosyl Radical-Dinuclear Iron Cluster Cofactor of *E. coli* Ribonucleotide Reductase."

Undergraduate — **Bachelor of Science** with distinction, Chemistry, 1986
The Pennsylvania State University (1982-1986)

PROFESSIONAL EXPERIENCE

- Russell and Mildred Marker Professor of Natural Products Chemistry — 2021-present
  The Pennsylvania State University
- Professor, Department of Chemistry — 2008-present
  The Pennsylvania State University
- Professor, Department of Biochemistry and Molecular Biology — 2008-present
  The Pennsylvania State University
- Associate Professor, Department of Chemistry, — 2004-2008
  The Pennsylvania State University
- Associate Professor, Department of Biochemistry and Molecular Biology, The Pennsylvania State University — 2002-2008
- Assistant Professor, Department of Biochemistry and Molecular Biology, The Pennsylvania State University — 1995-2002

AWARDS

- ACS BIOL Abeles and Jencks Award for the Chemistry of Biological Processes — 2023
- ACS Nobel Laureate Signature Award for Graduate Education in Chemistry — 2023
- American Society for Biochemistry and Molecular Biology William C. Rose Award — 2022
- Penn State Eberly College of Science Distinguished Faculty Mentoring Award — 2021
- Howard B. Palmer Faculty Mentoring Award — 2012
- Fellow, American Association for the Advancement of Science — 2010
- Penn State University Faculty Scholar Medal for Outstanding Achievement — 2009

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- Society for Biological Inorganic Chemistry Early Career Award 2008
- Searle Scholar Award of the Chicago Community Trust 1996-1999
- Camille and Henry Dreyfus New Faculty Award 1995
- National Institutes of Health Post-Doctoral Fellowship 1993-1994
- Whitaker Health Sciences Fund Fellowship 1989-1991

EDITORIAL ADVISORY BOARDS

- *Journal of Biological Chemistry* 2007-2011
- *Journal of Biological Inorganic Chemistry* 2007-2012
- *Biochemistry* 2013-2015

PROFESSIONAL MEMBERSHIPS

- American Association for the Advancement of Science
- American Chemical Society
- American Society for Biochemistry and Molecular Biology
- Society of Biological Inorganic Chemistry

OFFICES IN PROFESSIONAL ORGANIZATIONS

- Program Committee, American Chemical Society, Division of Biological Chemistry 2008-2012
- Co-chair of the Program Committee, ACS, Division of Biological Chemistry 2010-2012

RESEARCH INTERESTS

- Enzyme structures and reaction mechanisms
- Oxygen and C-H activation by metalloenzymes
- Control of high-valent metalloenzyme intermediates
- Design of enzyme inhibitors and inactivators
- Oxidation reactions in natural product biosynthesis
- Iron and oxygen homeostasis
- Epigenetic regulation of transcription by redox metalloenzymes
- Oxidations in neurotransmitter and bioactive peptide biosynthesis

ACTIVE RESEARCH SUPPORT

- Department of Energy DE-SC0016255, Production of Ethylene and 3-Hydroxypropanoate from the Common Metabolite, 2-Oxoglutarate, by the Ethylene-Forming Enzyme (EFE), March 1, 2023 to February 28, 2026 (M,P.I. with Carsten Krebs, Amie Boal, and Elvira Sayfutyarova)
- National Science Foundation 2108583, Exploration of the chemical and mechanistic diversity of mixed-valent iron oxygenases and oxidases, October 1, 2021 to September 30, 2024 (M.P.I. with Carsten Krebs and Alexey Silakov)
- National Institutes of Health R01 GM132653-02, Diverse Transition-Metal and Free-Radical Chemistry Enabling 2'-Deoxyribonucleotide Production by Bacteria in Restrictive Environments, June 1, 2019 to May 31, 2024 (P.I.)
- National Institutes of Health R01 GM138580-01, Structures and Mechanisms of “Heme-oxygenase-like” Non-heme Di-iron Enzymes that Catalyze Complex N-oxygenation and Olefin-installing C–C-Fragmentation Reactions, July 10, 2020 to June 30, 2024 (P.I.)

COMPLETED RESEARCH SUPPORT

- The Camille and Henry Dreyfus Foundation, Mechanisms and Consequences of Iron-Sulfur Cluster Assembly and Disassembly in Enzymes and Regulatory Proteins, July, 1995 to June, 2000 (P.I.)
- Searle Scholars/Chicago Community Trust, Mechanisms and Consequences of Iron-Sulfur Cluster Assembly and Disassembly in Enzymes and Regulatory Proteins, July 1, 1996 to June 30, 2000 (P.I.)

- National Institutes of Health R01 GM55365-01-04, Ribonucleotide Reductase: Iron-Radical Cofactor Assembly, January 1, 1997 to December 31, 2000 (P.I.)
- National Science Foundation, Structure-Function and Physiology of Novel Enzymes from Diverse Microbes, Research and Training Grant with J. G. Ferry, P. I., August 15, 1996 to August 14, 2001
- Tobacco Settlement Formula Fund, Mechanisms of Iron-Sulfur Cluster Assembly, July 1, 2002 to June 30, 2003 (co-P.I. with Carsten Krebs)
- Innovative Biotechnology Fund, Huck Institutes for the Life Sciences, Penn State University, The Mechanism of Oxygen Activation by Taurine/α-Ketoglutarate Dioxygenase, June 1, 2002 to May 31, 2004 (co-P.I. with Carsten Krebs)
- National Institutes of Health R01 GM55365, Ribonucleotide Reductase: Iron-Radical Cofactor Assembly, January 1, 2001 to December 31, 2005 (P.I.)
- National Science Foundation MCB-0235979, Structure, Mechanism, and Physiology of Two Sulfur-Mobilizing Enzymes from Synechocystis 6803, April 1, 2003 to March 30, 2006 (P.I.)
- Johnson and Johnson and Huck Institutes for the Life Sciences Innovative Biotechnology Seed Grant, Mechanism, Kinetics, and Inter-protein Interactions of Myo-Inositol Oxygenase, a Target for Treatment of Complications from Diabetes, July 1, 2005 to June 30, 2006 (co-P.I. with K. Sandeep Prabhu)
- National Institutes of Health R01 GM069657, Mechanism of Taurine:α-Ketoglutarate Dioxygenase, January 1, 2004 to December 31, 2009 (co-investigator; P.I. Carsten Krebs)
- National Science Foundation MCB-0642058, Non-Heme Fe(IV)-oxo Intermediates: Structure and Function, July 1, 2007 to June 30, 2010 (co-P.I. with Carsten Krebs)
- American Chemical Society, Petroleum Research Fund – Type AC grant Oxygen Activation by Isopenicillin N Synthase, September 1, 2007 to August 31, 2010 (co-P.I. with Carsten Krebs)
- National Science Foundation CHE-0724084, An Integrative Approach to Metalloenzyme-Catalyzed C-H Activation (joint program with the Deutsche Forschungsgesesellschaft), January 1, 2008 to December 31, 2010 (co-P.I. with Carsten Krebs and Sharon Hammes-Schiffer)
- National Institutes of Health R01 DK074641, Structure and Mechanism of myo-Inositol Oxygenase, February 1, 2007 to January 31, 2012 (P.I.)
- National Institutes of Health, Administrative Supplement to GM55365 Oxygen Activation and Radical Transfer in Ribonucleotide Reductase from Pathogens, June 1, 2010 to May 31, 2012 (lead P.I.; multiple P.I.s Carsten Krebs and Michael T. Green)
- National Institutes of Health R01 GM55365, Oxygen Activation and Radical Transfer in Ribonucleotide Reductase from Pathogens, August 15, 2008 to July 31, 2013 (lead P.I.; multiple P.I.s Carsten Krebs and Michael T. Green)
- National Science Foundation CHE-1058931, Structure and Function of Intermediates Involved in Metalloenzymatic N-Oxygenation and Oxidation Reactions, February 15, 2011 to January 31, 2015 (co-P.I. with Carsten Krebs)
- National Science Foundation MCB-1122079, Identification of the Dinuclear Metallo-Cofactor and Elucidation of the Reaction Mechanism of the Aldehyde Decarbonylase from the Alkane-Producing Cyanobacterium, Nostoc punctiforme, July 1, 2011 to June 30, 2014 (co-P.I. with Carsten Krebs and Squire Booker)
- Industrial – LS9 Inc., South San Francisco, CA, Fundamental Biochemistry and Mechanism of Aldehyde Decarbonylase in Conjunction with Acyl-ACP Reductase in the Synthesis of Alkanes, Olefins, and Reaction Byproducts, one-time unrestricted gift on December 1, 2011.
- National Institutes of Health R01 GM103268, Functionalization of Unactivated sp2-hybridized Carbon Atoms, July 1, 2012 to June 30, 2017 (M.P.I. with Squire Booker and Carsten Krebs)
- National Science Foundation MCB-1330784, Mapping the Hydrolase-oxygenase Boundaries and Range of Catalytic Capabilities in the HD-domain Dinuclear Metalloenzyme Superfamily, August 1, 2013 to July 31, 2017 (M.P.I with Carsten Krebs)
- National Institutes of Health R01 GM069657-06, Mechanism of Taurine: alpha-Ketoglutarate Dioxygenase, July 1, 2013 to June 30, 2018 (M.P.I. with Carsten Krebs)
- National Science Foundation CHE-1610676, Structures and Mechanisms of Two Enzymes that Produce Hydrocarbon Fuels from Abundant Metabolites, September 1, 2016 to August 31, 2019 (M.P.I. with Carsten Krebs and Amie Boal)

- National Institutes of Health R01 GM113106, Mechanisms of oxacycle- and olefin-installing iron/2-(oxo)glutarate oxygenases, September 10, 2015 to June 30, 2020 (M.P.I. with Carsten Krebs)
- National Institutes of Health R01 GM118812-05, Mechanisms and Reprogramming of Iron/2-Oxoglutarate Desaturases and Oxacyclases, June 1, 2016 to May 31, 2021 (M.P.I. with Carsten Krebs)
- Department of Energy DE-SC0016255, Mechanism of Ethylene Production from the Common Metabolite, 2-Oxoglutarate, by the Ethylene-Forming Enzyme (EFE), September 1, 2016 to August 31, 2021 (M.P.I. with Carsten Krebs and Amie Boal)
- Department of Energy DE-SC0016255, Mechanism of Ethylene Production from the Common Metabolite, 2-Oxoglutarate, by the Ethylene-Forming Enzyme (EFE), September 1, 2021 to February 28, 2023 (M.P.I. with Carsten Krebs and Amie Boal)

PUBLICATIONS

Refereed Original Research Articles

140) Burke, E. J.; Copeland, R. A.; Dixit, Y.; Krebs, C.; and **Bollinger, J. M., Jr.** "Steric Perturbation of the Grob-like Final Step of Ethylene-Forming Enzyme Enables 3-Hydroxypropionate and Propylene Production," *J. Am. Chem. Soc.* **2024**, *146*, 1977–1983.

139) Slater, J. W.; Neugebauer, M. E.; McBride, M. J.; Sil, D.; Lin, C. Y.; Katch, B. J.; Boal, A. K.; Chang, M. C. Y.; Silakov, A.; Krebs, C.; **Bollinger, J. M., Jr.** "Synergistic Binding of the Halide and Cationic Prime Substrate of the L-Lysine 4-Chlorinase, BesD, in Both Ferrous and Ferryl States," *Biochemistry* **2023**, 62, 2480-2491.

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3) Baker, C.H.; Banzon, J.; **Bollinger, J.M.**, Jr.; Stubbe, J.; Samano, V.; Robins, M.J.; Lippert, B.; Jarvi, E.; and Resvick, R. "2'-Deoxy-2'-methylenecytidine and 2'-Deoxy-2',2'-difluorocytidine 5'-Diphosphates: Potent Mechanism-Based Inhibitors of Ribonucleotide Reductase," *J. Med. Chem.,* **1991***,* 34, 1879-1884.

2) McCormick, J.M.; Reem, R.C.; Foroughi, J.; **Bollinger, J.M., Jr.;** Jensen, G. M.; Stephens, P.J.; Stubbe, J.; and Solomon, E.I. "Excited State Spectral Features of the Radical Reduced, Native and Fully Reduced Forms of the Coupled Binuclear Non-Heme Iron Center in Ribonucleotide Reductase: Active Site Differences Relative to Hemerythrin," *New J. Chem.,* **1991***,* 15, 439-444.

1) Mao, S.S.; Johnston, M.I.; **Bollinger, J.M., Jr.;** and Stubbe, J. "Mechanism-based inhibition of a mutant *Escherichia coli* ribonucleotide reductase (cysteine-225 > serine) by its substrate CDP," *Proc. Nat. Acad. Sci. USA*, **1989**, 86, 1485-1489.

Invited and Refereed Review Articles

11) Krebs, C.; Dassama, L.M.K.; Matthews, M.L.; Jiang, W.; Price, J.C.; Korboukh, V.; Li, N.; **Bollinger, J.M., Jr.** "Novel Approaches for the Accumulation of Oxygenated Intermediates to Multi-Millimolar Concentrations," *Coord. Chem. Rev.,* **2013**, 257, 234-243

10) Krebs, C.; **Bollinger, J. M. Jr.**; Booker, S. J. "Cyanobacterial alkane biosynthesis further expands the catalytic repertoire of the ferritin-like 'di-iron-carboxylate' proteins," *Curr. Opin. Chem. Biol.*, **2011**, *15*, 291-303.

9) van der Donk, W. A.; Krebs, C.; **Bollinger, J. M., Jr.** "Substrate Activation by Iron Superoxo Intermediates," *Curr. Opin. Struct. Biol.,* **2010**, *20*, 673-683.

8) **Bollinger, J.M., Jr.;** Diao, Y.; Matthews, M.; Xing, G.; Krebs, C. **"***myo*-Inositol Oxygenase: a Radical New Pathway for O_2 and C-H Activation at a Nonheme Diiron Cluster," *Dalton Trans.*, **2009**, *2009*, 905-914.

7) Jiang, W.; Saleh, L.; Yun, D. **Bollinger, J.M., Jr.;** Krebs, C. "Formation and Function of the Manganese(IV)/Iron(III) Cofactor in *Chlamydia trachomatis* Ribonucleotide Reductase," *Biochemistry*, **2008**, *47*, 13736-13744.

6) **Bollinger, J.M., Jr.;** Jiang, W.; Green, M. T.; Krebs, C. "The Manganese(IV)/Iron(III) Cofactor of *Chlamydia trachomatis* Ribonucleotide Reductase: Structure, Assembly, Radical Initiation, and Evolution," *Curr. Opin. Struct. Biol.*, **2008**, *18*, 650-657.

5) Krebs, C., Galonic, D.; Walsh, C. T.; **Bollinger, J. M., Jr.** "Non-Heme Fe(IV)-Oxo Intermediates," *Acc. Chem. Res.*, **2007**, *40*, 484-492.

4) **Bollinger, J. M., Jr.**, Krebs, C. "Enzymatic C-H-Activation by Metal-Superoxo Complexes," *Curr. Opin. Chem. Biol.* **2007***, 11*, 151-158.

3) **Bollinger, J. M., Jr.,** Krebs, C. "Stalking Intermediates in Oxygen Activation by Iron Enzymes: Motivation and Method," *J. Inorg. Biochem.,* **2006***, 100*, 586-605.

2) **Bollinger, J. M., Jr.**, Price, J. C., Hoffart, L. M., Barr, E. W., Krebs, C. "Mechanism of Taurine:α-Ketoglutarate (TauD) from *Escherichia coli*," *Eur. J. Inorg. Chem.*, **2005**, 4245-4254.

1) Krebs, C., Price, J. C., Baldwin, J., Saleh, L., Green, M. T., and **Bollinger, J. M., Jr.** "Rapid Freeze-Quench ^{57}Fe-Mössbauer Spectroscopy: Monitoring Changes of an Iron-containing Active Site during a Biochemical Reaction," *Inorg. Chem.* **2005**, *44*, 742-757.

Invited Perspectives

5) Boal, A. K.; **Bollinger, J. M., Jr.**; Chang, W.-c. "Assembly of the Unusual Oxacycles in the Orthosomycin Antibiotics," *Proc. Natl. Acad. Sci. U.S.A.*, **2015**, *112*, 11989-11990.

4) **Bollinger, J. M. Jr.**, "Biochemistry: Getting the Metal Right," *Nature*, **2010**, *465*, 40-41.

3) **Bollinger, J. M., Jr.**, Matthews, M. L. "Biochemistry: Remote Enzyme Microsurgery," *Science*, **2010**, 327, 1337-1338.

2) **Bollinger, J. M., Jr.**, Broderick, J.B. "Frontiers in enzymatic C- H bond activation," Editorial Overview in *Current Opinion in Chemical Biology*, **2009**, *13*, 51-57.

1) **Bollinger, J. M., Jr.**, "Biochemistry: Electron Relay in Proteins," *Science,* **2008**, *320*, 1730-1731.

Invited Review Articles

1) **Bollinger, J. M., Jr.**, Tong, W. H., Ravi, N., Huynh, B. H., Edmondson, D. E., and Stubbe, J. "Use of Rapid Kinetics Methods to Study the Assembly of the Diferric-Tyrosyl Radical Cofactor of *E. coli* Ribonucleotide Reductase," *Meth. Enzymol.,* **1995***, 258*, 278-303.

Book Chapters

3) Rajakovich, L. R.; Zhang, B.; McBride, M. J.; Boal, A. K.; Krebs, C.; **Bollinger, J.M., Jr.** (2020) "Emerging Structural and Functional Diversity in Proteins with Dioxygen-Reactive Dinuclear Transition Metal Cofactors," In: Hung-Wen (Ben) Liu, Tadhg P. Begley (eds.) Comprehensive Natural Products III: Chemistry and Biology, *5*, 215-250, Elsevier.

2) **Bollinger, J.M., Jr.;** Chang, W-c.; Matthews, M.L.; Martinie, R.; Boal, A.K.; Krebs, C. (2015) "Mechanisms of 2-Oxoglutarate-Dependent Oxygenases: The Hydroxylation Paradigm and Beyond" in *RSC Metallobiology Series No. 3*. R.P. Hausinger and C.J. Schofield, eds. pp. 95-122. Royal Society of Chemistry, Washington, D.C.

1) Huynh, B. H.; **Bollinger, J. M., Jr.**; and Edmondson, D. E. (1998) "Reaction intermediates in oxygen activation by enzymes containing carboxylate-bridged binuclear iron clusters" in *Spectroscopic Methods in Bioinorganic Chemistry,* E. I Solomon and K. O. Hodgson, eds. pp. 403-422. American Chemical Society, Washington, D.C.

INVITED LECTURES

At Regional, National and International Meetings

1) "Control of the Outcome of Oxygen Activation by the Diiron-Carboxylate Proteins," **Annual Conference on Enzymes in Deoxyribonucleotide Synthesis**, September 4-7, 1998, Sandhamn, Sweden.

2) "Structural Determinants of the Mechanisms and Outcomes of O_2 Activation by the Diiron Carboxylate Proteins," 1999 **International Conference on Biological Inorganic Chemistry**, July 11-16, 1999, Minneapolis, MN. Published abstract: *J. Inorg. Biochem.* 1999, *74*, 13.

3) "An Fe(IV) Intermediate in the Reaction of Taurine/Alpha-ketoglutarate Dioxygenase from *E. coli*," 2003 **International Conference on Biological Inorganic Chemistry**, July 19-23, 2003, Cairns, Australia. Published abstract: *J. Inorg. Biochem.* 2003, *96*, 223.

4) "Identification of the Precursor to Cluster X in Oxygen Activation by the Diiron(II) Center in Protein R2 of Ribonucleotide Reductase from *Escherichia coli*." **227th ACS National Meeting**, March 28-April 1, 2004, Anaheim, CA. Published abstract Abstracts of Papers, 2004, INOR-805.

5) "Precursors to Cluster **X** in Oxygen Activation by the Diiron(II) Cluster in Protein R2 of Ribonucleotide Reductase from *Escherichia coli*." **19th Enzyme Mechanisms Conference**, January 5-9, 2005, Pacific Grove, CA.

6) "New Tricks from an Old Dog: Novel Mechanisms of Oxygen Activation and C-H Cleavage by a Dinuclear Iron Cluster." **Gordon Research Conference on Protein Derived Cofactors, Radicals and Quinones**, January 22-27, 2006, Ventura, CA (cancelled due to family medical emergency).

7) "New Tricks from an Old Dog: Novel Mechanisms of Oxygen Activation and C-H Cleavage by a Dinuclear Iron Cluster." **Gordon Research Conference on Metals in Biology**, January 29-February 3, 2006, Ventura, CA (cancelled due to family medical emergency).

8) "Novel Mechanisms of Oxygen Activation and C-H Cleavage by a Dinuclear Iron Cluster." **German Research Foundation Meeting on Radicals in Enzymatic Catalysis**, March 6-8, 2006, Marburg, Germany (cancelled due to family medical emergency).

9) "Oxygen activation by a mixed-valent diiron cluster and C-H cleavage by an iron-superoxide intermediate in the glycol cleavage reaction catalyzed by *myo*-inositol oxygenase" **25th Summer Symposium in Molecular Biology: Frontiers in Metallobiochemistry**, June 7-10, 2006, University Park, PA

10) "Mechanism of Protein and Substrate Radical Generation in the Class Ic Ribonucleotide Reductase from *Chlamydia trachomatis*" **Gordon Research Conference on Metals in Biology**, January 28-February 2, 2007, Ventura, CA

11) "Formation and Function of a Radical-Initiating Mn(IV)/Fe(III) Cofactor in Ribonucleotide Reductase from *Chlamydia trachomatis*" **Gordon Research Conference on Enzymes, Co-enzymes, and Metabolic Pathways**, July 8-13, 2007, Biddeford, ME

12) "Formation and Function of a Radical-Initiating Mn(IV)/Fe(III) Cofactor in Ribonucleotide Reductase from *Chlamydia trachomatis*" **Gordon Research Conference on Protein Cofactors, Radicals And Quinones**, January 20-25, 2008, Ventura, CA

13) "Stabilization of Fe(IV)-oxo Intermediates in Proteins: Tactics and Limits" **Pre-ICBIC14 Symposium on Dioxygen Activation by Metalloenzymes and Models**, March 20-21, 2008, Nagoya, Japan (cancelled for health reasons)

14) "Strategies for and Intermediates in O_2 and C-H Activation by Iron Enzymes" **235th National Meeting and Exposition of the American Chemical Society** (Alfred Bader Award Symposium for Lawrence Que, Jr.), April 6-10, 2008, New Orleans, LA

15) "Mechanistic and Evolutionary Aspects of Radical Initiation by the Mn(IV)/Fe(III) Cofactor of Chlamydia trachomatis Ribonucleotide Reductase," **21st Enzyme Mechanisms Conference Loews Ventana Canyon Resort**, January 3-6, 2009, Tucson, AZ

16) "Why the iron- and ?-ketoglutarate-dependent aliphatic halogenases are not hydroxylases," Plenary Lecture in Honor of 2008 Society for Biological Inorganic Chemistry Early Career Award, **14th International Conference on Biological Inorganic Chemistry**, July 25-30, 2009, Nagoya, Japan

17) "How the Aliphatic Halogenases Avoid Competing Hydroxylation," **24th Annual Symposium of The Protein Society**, August 1-5, 2010, San Diego, CA

18) "How the Aliphatic Halogenases Avoid Competing Hydroxylation," **The 2010 International Chemical Congress of Pacific Basin Societies (Pacifichem)**, December 15-20, 2010, Honolulu, HI (sent student, Megan Matthews, in my place due to family health emergency)

19) "The Mn/Fe Redox Cofactor of Chlamydia trachomatis Ribonucleotide Reductase: One of a Kind, or the First of Many?" **Gordon Research Conference on Inorganic Reaction Mechanisms**, March 6-11, 2011, Galveston, TX

20) "Cyanobacterial Aldehyde Decarbonylase Expands the Catalytic Repertoire of the Ferritin-like Dimetal Carboxylate Proteins," **Gordon Research Conference on Enzymes, Coenzymes, and Metabolic Pathways**, July 10-15, 2011, Waterville Valley, NH

21) "Cyanobacterial Aldehyde Decarbonylase: A Surprising New Catalytic Capability for the Ferritin-Like Dimetal-Carboxylate Proteins," **Society for Industrial Microbiology (SIM) Annual Meeting and Exposition**, July 24-28, 2011, New Orleans, LA

22) "Protein-Gated, Intersubunit, Long-Distance Hole Transfer (PCET) in the Manganese- and Iron-Dependent Class Ic Ribonucleotide Reductase from *Chlamydia trachomatis*,"**1st International Conference on Proton-Coupled Electron Transfers: PCET, from Biology to Catalysis**," October 9-13, 2011, Loire Valley, France

23) "Cyanobacterial Aldehyde Decarbonylase Expands the Catalytic Repertoire of the Ferritin-like Dimetal Carboxylate Proteins," **Zing Conference on Enzymes, Coenzymes, and Metabolic Pathways**, November 17-21, 2011, Xcaret, Mexico (sent Ph.D. student Ning Li due to family medical emergency)

24) "The Novel, Cryptically Redox Monooxygenation Reaction Catalyzed by Cyanobacterial Aldehyde Decarbonylase and Evidence Against the Proposed Dioxygen-Independent Pathway," **Gordon Research Conference on Isotopes in Biological and Chemical Sciences**, February 5-10, 2012, Galveston, TX (sent Prof. Carsten Krebs in my place due to family medical emergency)

25) "Dissection of Mechanisms of O_2-Utilizing Metalloenzymes by Direct Observation of Reactive Intermediates," **243rd National Meeting and Exposition of the American Chemical Society** (Special Symposium on Electronic Structure in Honor of Edward I. Solomon), March 25-29, 2012, San Diego, CA

26) "O_2-Evolving Chlorite Dismutase as a Tool for Studying O_2-Utilizing Enzymes and Selected Applications," **Gordon Research Conference on Protein Cofactors, Radicals and Quinones**, July 29 – August 3, 2012, Mount Holyoke College, Holyoke, MA.

27) "Mechanism of the Aliphatic Halogenases," **Society of Industrial Microbiology and Biotechnology Annual National Meeting and Exposition,** August 12-15, 2012, Washington, DC (sent Dr. Megan Matthews in my place due to a family medical emergency)

28) "The Mechanism of the Cryptically-Redox Oxygenation Reaction Catalyzed by Aldehyde Deformyl-ating Oxygenase (Formerly Aldehyde Decarbonylase)," **Gordon Research Conference on Metals in Biology**, January 20 – 25, 2013, Ventura, CA.

29) "The Mechanism of the Cryptically-Redox Oxygenation Reaction Catalyzed by Aldehyde Deformyl-ating Oxygenase (Formerly Aldehyde Decarbonylase)," **245th American Chemical Society National Meeting,** April 7 – 11, 2013, New Orleans, LA.

30) "Non-haem Iron Enzymes in the Biosyntheses of Bioactive Natural Products," **International Conference on Bioinorganic Chemistry**, July 22 – 26, 2013, Grenoble, France (sent Dr. W.-c. Chang in my place due to a family medical emergency).

31) "Mapping the Hydrolase-oxygenase Boundaries and Range of Catalytic Capabilities in the HD-domain Dinuclear Metalloenzyme Superfamily," **23rd Solvay Conference on Chemistry**, October 16 – 19, 2013, Brussels, Belgium (cancelled due to a family medical emergency).

32) "Fatty Acids to Diesel Fuel: the Mechanism of the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," **247th American Chemical Society National Meeting**, March 16-20, 2014, Dallas, TX.

33) "Frontiers Beyond the Hydroxylation Paradigm in the Diverse Chemisty of Non-heme Fe(IV)-oxo (Ferryl) Enzyme Intermediates," **2014 ASBMB Annual Meeting**, April 26-30, 2013, San Diego, CA.

34) "Demystifying the Chemical Magic of Non-heme-iron Enzymes in Natural Product Biosynthesis," **2014 Gordon Research Conference on Iron-Sulfur Enzymes**, June 15-20, 2014, Easton, MA.

35) "The Remarkable Versatility of Hydrogen-Abstracting Iron(IV)-oxo (Ferryl) Enzyme Intermediates in the Biosynthesis of Bioactive Natural Products," **International Conference on Hydrogen Atom Transfer**, June 22-26, 2014, Rome, Italy.

36) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," **2014 Gordon Research Conference on Enzymes, Coenzyme, and Metabolic Pathways**, July 13-18, 2014, Waterville Valley, NH.

37) "Synthesis of Natural-Product Drugs by Iron and 2-(Oxo)glutarate Oxygenases," **2015 GTC Conference on Enzymes in Drug Discovery**, February 26-27, 2015, San Diego, CA.

38) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes in the Biosynthesis of Bioactive Natural Products," **2015 Southeast Enzymes Meeting**, April 11, 2015, Atlanta, GA.

39) "Fatty Acids to Diesel Fuel: the Mechanism of the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," **Pacifichem 2015**, December 15-20, 2015, Honolulu, HI.

40) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," **Pacifichem 2015**, December 15-20, 2015, Honolulu, HI.

41) "Mechanisms and Purview of C-H-Bond Activation by Iron(III)-Superoxide Complexes," **251st American Chemical Society National Meeting**, March 12-16, 2016, San Diego, CA.

42) "Mechanism and Outcome Control in the Petrodiesel-Producing Diiron Enzyme, Aldehyde Deformylating Oxygenase," **A Sojourn in Biochemistry: RNR (R&R) Was Anything But (symposium to honor JoAnne Stubbe's 70th birthday and career)**, June 11-12, 2016, Cambridge, MA

43) "Mechanism and Reaction Control in the Petrodiesel-Producing Diiron Enzyme, Aldehyde Deformylating Oxygenase," **5th Symposium for Advanced Biological Inorganic Chemistry**, January 7-11, 2017, Kolkata, India.

44) "Emerging Diversity in the Chemistry of Non-Heme Diiron Oxidases and Oxygenases," **Gordon Research Conference on Metals in Biology**, January 22-27, 2017, Ventura, CA.

45) "Novel Outcomes and Mechanisms Emerging from Dioxygen Activation by Non-Heme Di-Iron Enzymes," **253rd American Chemical Society National Meeting**, April 2-6, 2017, San Francisco, CA.

46) "Mechanistic Pathways to Unusual Outcomes in Reactions of Iron-Dependent Oxygenases," "**Experimental Biology 2017"** Symposium of the **2017 Annual Meeting of the American Society of Biochemistry & Molecular Biology**, April 22-26, 2017, San Francisco, CA.

47) "Capabilities and Limitations of Mid-Valent Iron-Superoxide Complexes for Hydrogen Atom Transfer," **International Conference on Hydrogen Atom Transfer**, July 2-6, 2017, Rome, Italy.

48) "How Iron- and 2-(Oxo)glutarate-dependent (Fe/2OG) Oxygenases Direct Their Common Ferryl Intermediate to Different Outcomes," **26th Enzyme Mechanisms Conference**, January 6-9, 2019, New Orleans, LA

49) Title to be determined, **19th International Conference on Biological Inorganic Chemistry**, August 11-16, 2019, Interlaken, Switzerland (withdrew for health reasons)

50) "Mechanistic and Structural Analysis of How Nonheme-Iron Enzymes Direct Different Oxidation Reactions," **258th American Chemical Society National Meeting**, August 25-29, 2019, San Diego, CA

51) Title to be determined, **International Conference on Hydrogen Atom Transfer**, June 21-25, 2020, Rome, Italy (cancelled due to Covid-19 pandemic).

52) **"**How Nonheme-Iron Enzymes Achieve Such Remarkable Versatility in Biological C–H Bond Activation" (keynote lecture), **Midwest Enzyme Chemistry Conference**, October 24, 2020, remote.

53) Plenary Lecture, **EuroBIC**, July 17-22, 2022, Grenoble France (declined due to Covid-19 risk)

54) "Versatile Catalytic Capabilities and Novel Functional Paradigm of the Heme-Oxygenase-Like Diiron Oxygenases and Oxidases (HDOs)," **2023 Alfred Bader Award Symposium in Honor of R. David Britt, American Chemical Society National Meeting**, March 26-30, 2023, Indianapolis, IN (presented remotely)

55) "Radical-Polar Crossover Mechanism of Ethylene-Forming Enzyme (EFE) and Perturbation of its Cryptic Second Branch Point," **Symposium in Celebration of 2023 Nobel Laureate Signature Award for Graduate Education in Chemistry to Rachelle Copeland, Carsten Krebs, and J. Martin Bollinger, Jr., American Chemical Society National Meeting**, March 26-30, 2023 Indianapolis, IN (presented remotely)

56) "Control of Reaction Pathways and Outcomes by Iron(II)- and 2-Oxoglutarate-Dependent Oxygenases," **2023 ACS BIOL Abeles and Jencks Award Symposium in Honor of J. Martin Bollinger, Jr., American Chemical Society National Meeting**, August 13-17, 2023 San Francisco, CA

57) Title to be determined, **International Conference on Hydrogen Atom Transfer**, June 9-13, 2024, Monteporzio Catone, Italy.

At Companies

1) "Mutagenesis to Probe Protein Control of Dioxygen Activation at the Diiron Cluster in Protein R2 of Ribonucleotide Reductase," 3M Corporation, Minneapolis, MN, June 21, 1996

2) "Why the Iron- and ?-Ketoglutarate-dependent Aliphatic Halogenases are not Hydroxylases," New England Biolabs, Ipswich, MA, October 8, 2009

3) "Cyanobacterial Aldehyde Decarbonylase Expands the Catalytic Repertoire of the Ferritin-like Dimetal Carboxylate Proteins," LS9, Inc., South San Francisco, CA, June 17, 2011

4) "Monooxygenases and Dioxygenases: Nomenclature and General Principles," Orrick, Herrington, and Sutcliffe, LLP, San Francisco, CA, September 27, 2011

5) "The Mechanism of the Cryptically-Redox Oxygenation Reaction Catalyzed by Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," New England Biolabs, Ipswich, MA, June 27, 2013.

6) "Cyanobacterial Conversion of Abundant Fatty Acids to Petrodiesel via the Cryptically Redox Reaction of Aldehyde Deformylating Oxygenase," ExxonMobil Research and Engineering Company, Annandale, NJ, May 21, 2018.

At Universities

1) "Control of the Outcome of Oxygen Activation by the Diiron-Carboxylate Proteins," Program in Molecular Medicine, Penn State University College of Medicine, October 15, 1997

2) "Control of the Outcome of Oxygen Activation by the Diiron-Carboxylate Proteins," Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, November 7, 1997

3) "Control of the Outcome of Oxygen Activation by the Diiron-Carboxylate Proteins," Department of Chemistry, Duquesne University, January 30, 1998

4) "Structural Determinants of Reaction Mechanism and Outcome In Oxygen Activation by the Di-Iron Proteins," Department of Biophysics, Johns Hopkins University, May 4, 1998

5) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Departments of Chemistry and Physics, Emory University, September 29, 1998

6) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Biochemistry, Temple University School of Medicine, October 9, 1998

7) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Biochemistry, McMaster University (Canada) October 27, 1998

8) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Chemistry, Northwestern University, November 13, 1998

9) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Chemistry, University of Minnesota, December 14, 1998

10) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Interdepartmental Program in Medicinal Chemistry, University of Michigan, January 28, 1999

11) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Chemistry, Stanford University, February 2, 1999

12) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Chemistry, Oregon Graduate Institute, February 4, 1999

13) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Biochemistry and Biophysics, Oregon State University, February 5, 1999

14) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Biochemistry and Biophysics, University of Pennsylvania, "George Raiziss Biochemical Rounds: Enzymology at the Interface of Chemistry and Medicine" September 14, 1999

15) "Structural Determinants of Reaction Outcome in Oxygen Activation by the Di-Iron-Carboxylate Proteins," Department of Biochemistry, University of Wisconsin, November 1, 1999

16) "Structural and Mechanistic Bases for the Divergent Outcomes of Dioxygen Activation by the Diiron-Carboxylate Oxygenase and Oxidase Proteins," Department of Chemistry, Ohio State University, January 10, 2000

17) "Structural and Mechanistic Bases for the Divergent Outcomes of Dioxygen Activation by the Diiron-Carboxylate Oxygenase and Oxidase Proteins," Department of Chemistry, Columbia University, January 20, 2000

18) "Structural and Mechanistic Bases for the Divergent Outcomes of Dioxygen Activation by the Diiron-Carboxylate Oxygenase and Oxidase Proteins," Department of Molecular Biology, The Scripps Research Institute, February 2, 2000

19) "Mechanism of Rapid Electron Transfer During Oxygen Activation at the Diiron-Carboxylate Center of Ribonucleotide Reductase Protein R2," Department of Biochemistry, Stockholm University (Sweden), September 21, 2000

20) "Aiming a Molecular Cannon: Protein Control of Di-Oxygen Activation at Carboxylate-Bridged Dinuclear Iron Clusters," Department of Chemistry, Penn State University, September 26, 2000

21) "Aiming a Molecular Cannon: Protein Control of Di-Oxygen Activation at Carboxylate-Bridged Dinuclear Iron Clusters," Department of Chemistry, University at Buffalo (SUNY), February 28, 2001

22) "Aiming a Molecular Cannon: Protein Control of Di-Oxygen Activation at Carboxylate-Bridged Dinuclear Iron Clusters," Department of Biochemistry, Virginia Polytechnical Institute and State University, September 10, 2001

23) "Aiming a Molecular Cannon: Protein Control of Di-Oxygen Activation at Carboxylate-Bridged Dinuclear Iron Clusters," Department of Chemistry, California Institute of Technology, October 22, 2001

24) "Probing the Structural and Mechanistic Bases for the Divergent Control of Oxygen Activation by the Diiron-Carboxylate Proteins." Department of Chemistry, Massachusetts Institute of Technology, December 3, 2001

25) "Aiming a Molecular Cannon: Protein Control of Di-Oxygen Activation at Carboxylate-Bridged Dinuclear Iron Clusters," Program in Complex Biological Systems, Montana State University, December 7, 2001

26) "Aiming a Molecular Cannon: Protein Control of Di-Oxygen Activation at Carboxylate-Bridged Dinuclear Iron Clusters," Department of Chemistry, Bucknell University, March 27, 2002

27) "Dissecting the Mechanism of Oxygen Activation by Taurine:▯-Ketoglutarate Dioxygenase," Williams College, September 26, 2003

28) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," Department of Chemistry, Utah State University, October 15, 2003

29) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," Department of Chemistry, Penn State University, November 11, 2003

30) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," Department of Chemistry, University of Minnesota, March 3, 2004

31) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," Department of Chemistry, Montana State University, April 30, 2004

32) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," University of Kansas, May 11, 2004

33) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," University of Pennsylvania, Department of Chemistry Inorganic Seminar Series, October 26, 2004

34) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," University of Delaware, Department of Chemistry, February 7, 2005

35) "Stalking Intermediates in Oxygen Activation by Iron Enzymes," Texas A. & M. University, Department of Chemistry, September 29, 2005

36) "Stalking Intermediates in Oxygen Activation by Iron Proteins," Duquesne University, Mini-symposium in convocation of their Center for Biological Inorganic Chemistry, November 4, 2005

37) "Novel Mechanism of *myo*-Inositol Oxygenase," University of Michigan, Department of Biological Chemistry, May 2, 2006

38) "Novel Mechanism of *myo*-Inositol Oxygenase," Emory University, Department of Chemistry, Biomedical Division, December 11, 2006

39) "Novel Mechanisms of O_2 and C-H Activation by a Diiron Cluster in *myo*-Inositol Oxygenase," King's College, April 4, 2007 (Lecture at the 369th meeting of the American Chemical Society Susequehanna Valley Section)

40) "Novel Mechanisms of O_2 and C-H Activation by a Diiron Cluster in *myo*-Inositol Oxygenase," Moravian College, April 11, 2007

41) "Bioinorganic Chemistry: the Coming Decade and Beyond," Emory University, June 13, 2007 (This was a three-hour "vision" presentation given jointly with Profs. Carsten Krebs, Squire Booker, and Michael Green)

42) "Dissecting Mechanisms of Metalloenzymes by Kinetics, Spectroscopy, and Protein and Substrate Mutagenesis," Department of Chemistry, Technical University of Denmark, Kgs. Lyngby, Denmark, June 19, 2007 (This was a three-hour tutorial delivered at a Ph. D. school as part of their Summer school program with expenses paid by the host.)

43) "Strategies for O_2 and C-H Activation by Metalloenzymes," R. B. Woodward Lectures in the Chemical Sciences, Harvard/M.I.T. Inorganic Chemistry Seminar, Harvard University, September 19-20, 2007

44) "Formation, function, and evolution of a radical-initiating manganese-iron cofactor in ribonucleotide reductase from *Chlamydia trachomatis*," Chemical Biology/Inorganic Chemistry Series, University of Illinois at Urbana-Champaign, November 29, 2007

45) "Formation and Function of a Radical Initiating Mn(IV)/Fe(III) Cofactor in the Ribonucleotide Reductase from *Chlamydia trachomatis,"* Department of Chemistry, University of California, Irvine, May 9, 2008

46) "Formation and function of a stable high-valent, manganese-iron redox cofactor in the ribonucleotide reductase from *Chlamydia trachomatis*," Department of Chemistry, Yale University, September 23, 2008 (postponed due to family medical emergency)

47) "The Mid-Valent Metal-Superoxo Manifold for Enzymatic O_2 and C-H Activation," University of Minnesota Chemical Biology Training Grant Symposium, May 27, 2009

48) "The Basis for the Strict Selectivity of Aliphatic Halogenases against Substrate Hydroxylation," Department of Chemistry, Princeton University, September 10, 2009

49) "Why the Iron- and Alpha-Ketoglutarate-Dependent Aliphatic Halogenases are not Hydroxylases," Department of Chemistry, Case Western Reserve University, October 1, 2009

50) "Cyanobacterial Aldehyde Decarbonylase Expands the Catalytic Repertoire of the Ferritin-like Dimetal Carboxylate Proteins," Department of Chemistry, University of California at Berkeley, September 26, 2011

51) "Cyanobacterial Aldehyde Decarbonylase Expands the Catalytic Repertoire of the Ferritin-like Dimetal Carboxylate Proteins," Department of Chemistry, Loyola University of Chicago, September 29, 2011

52) "Cyanobacterial Aldehyde Decarbonylase Expands the Catalytic Repertoire of the Ferritin-like Dimetal Carboxylate Proteins," Department of Chemistry, Carnegie Mellon University, November 17, 2011

53) "Cyanobacterial Aldehyde Decarbonylase: A Surprising New Catalytic Capability for the Ferritin-Like Dimetal-Carboxylate Proteins," Department of Biochemistry and Molecular Biology, Michigan State University, January 19, 2012

54) "Cyanobacterial Aldehyde Decarbonylase: A Surprising New Catalytic Capability for the Ferritin-Like Dimetal-Carboxylate Proteins," Department of Chemistry, University of Chicago, January 30, 2012

55) "Cyanobacterial Aldehyde Decarbonylase: A Surprising New Catalytic Capability for the Ferritin-Like Dimetal-Carboxylate Proteins," Department of Chemistry, University of Florida, February 20, 2012 (postponed due to family health emergency)

56) "Cyanobacterial Aldehyde Decarbonylase: A Surprising New Catalytic Capability for the Ferritin-Like Dimetal-Carboxylate Proteins," Department of Chemistry, Florida State University, February 21, 2012 (postponed due to family health emergency).

57) "Making Diesel Fuel in Bacteria: the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," Department of Chemistry, University of California at Davis, February 5, 2013.

58) "Making Diesel Fuel in Bacteria: the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," Department of Chemistry, Texas A&M, February 21, 2013 (postponed due to family health emergency)

59) "Making Diesel Fuel in Bacteria: the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," Department of Chemistry, Ohio State University, February 25, 2013 (postponed due to family health emergency).

60) "Making Diesel Fuel in Bacteria: the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," Department of Chemistry, Northwestern University, April 11, 2013.

61) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Departments of Chemistry and Biochemistry, University of Texas, Austin, February 28, 2014 (postponed due to family health emergency).

62) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Chemistry, University of California, San Diego, May 2, 2014 (postponed; rescheduled for January 9, 2015).

63) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," **2014 Baker Symposium**, Cornell University, May 2-3, 2014.

64) "Fatty Acids to Diesel Fuel: the Mechanism of the Cryptically Redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase (Formerly Aldehyde Decarbonylase)," Energy Biosciences Institute, University of California, Berkeley, September 30, 2014.

66) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Chemistry, University of California, San Diego, January 9, 2015.

67) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Chemistry, Wake Forest University, January 21, 2015.

68) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Chemistry, University of Minnesota, Twin Cities, February 12, 2015.

69) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Macalester College, Minneapolis, Minnesota, February 13, 2015.

70) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Chemistry, Brigham Young University, February 23, 2015.

71) "Making Diesel Fuel in Bacteria via the Cryptically-redox Oxygenation Reaction of Aldehyde Deformylating Oxygenase," Department of Chemistry, Texas A & M University, College Station, TX, March 4, 2015.

72) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Molecular Biosciences, University of Texas at Austin, Austin, TX, May 6, 2015.

73) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes in Biosynthetic Pathways to Natural Product Drugs," Molecular Life Sciences Seminar Series, The Ohio State University, Columbus, Ohio, February 16, 2016.

74) "Demystifying the Chemical Magic of Non-Heme-Iron Enzymes Involved in Biosynthesis of Bioactive Natural Products," Department of Chemistry, Princeton University, Princeton, NJ, March 24, 2016.

75) "The Remarkable Versatility of High-valent-iron Intermediates in Biosynthesis of Bioactive Natural Products," M.I.T Department of Chemistry T.Y. Shen Lectureship, Massachusetts Institute of Technology, Cambridge, MA, April 9, 2018.

76) "Unique Capabilities (and Limitations) of C–H-Activation by Iron-Superoxide Enzyme Intermediates," M.I.T Department of Chemistry T.Y. Shen Lectureship, Massachusetts Institute of Technology, Cambridge, MA, April 10, 2018.

77) "The Remarkable Versatility of High-Valent Iron Intermediates in Biosynthesis of Bioactive Natural Products" Departments of Chemistry and Pharmacy, University of Kentucky, Lexington, KY, April 13, 2018.

78) "The Remarkable Versatility of High-Valent Iron Intermediates in Biosynthesis of Bioactive Natural Products," Departments of Chemistry, North Carolina State University, Raleigh, NC, April 20, 2018.

79) "Capabilities and Limitations of Iron(III)-superoxo Intermediates in Enzymatic Transformation of Aliphatic Carbons," Department of Chemistry, University of Washington, Seattle, WA, January 28, 2020.

80) "Protein Control of Outcome in Activation of Dioxygen by Nonheme Iron Enzymes," Department of Chemistry, University of California, Santa Barbara, Santa Barbara, CA, March 05, 2024.

81) "Protein Control of Outcome in Activation of Dioxygen by Nonheme Iron Enzymes," Department of Chemistry, University of California, Irvine, Irvine, CA, March 07, 2024.

82) "Protein Control of Outcome in Activation of Dioxygen by Nonheme Iron Enzymes," Watanabe Symposium in Chemical Biology, University of Indiana, Bloomington IN, September 21, 2024 (invitation accepted).

OTHER PARTICIPATION IN INTERNATIONAL MEETINGS

1) Discussion Leader, Gordon Research Conference on Protein Cofactors, Radicals and Quinones, January 13-18, 2002, Ventura, CA.

2) Discussion Leader, Gordon Research Conference on Enzymes Coenzymes and Metabolic Pathways, July 21-26, 2002, Kimball Union Academy, NH (declined for family reasons).

3) Discussion Leader, Gordon Research Conference on Metals in Biology, January 27 - February 1, 2008, Ventura, CA.

4) Co-Vice-Chair, Gordon Research Conference on Enzymes, Coenzymes and Metabolic Pathways, July 13-18, 2008, Biddeford, ME.

5) Co-Chair, Gordon Research Conference on Enzymes Coenzymes and Metabolic Pathways, July 12-17, 2009, Waterville Valley, NH.

6) Discussion Leader, Gordon Research Conference on Protein Cofactors, Radicals and Quinones, January 24-29, 2010, Ventura, CA.

7) Co-Chair, 29th Annual Penn State Summer Symposium in Molecular Biology: Frontiers in Metallobiochemistry, June 1-5, 2010, University Park, PA

8) Chair, Division of Biological Chemistry Program of the American Chemical Society, Fall National Meeting, August 22-26, 2010, Boston, MA

9) Chair, Division of Biological Chemistry Program of the American Chemical Society, Spring National Meeting, March 27-31, 2011, Anaheim, CA

10) Discussion Leader, Gordon Research Conference on Protein Cofactors, Radicals and Quinones, July 29 - August 3, 2012, Holyoke, MA.

11) Co-Chair, 33rd Annual Penn State Summer Symposium in Molecular Biology: Frontiers in Metallobiochemistry, June 4-7, 2014, University Park, PA

12) Discussion Leader, Gordon Research Conference on Metals in Biology, January 24-29, 2016, Ventura, CA

13) Co-Chair, "A Sojourn in Biochemistry: RNR (R&R) Was Anything But" (symposium to honor JoAnne Stubbe's 70th birthday and career), June 11-12, 2016, Cambridge, MA

14) Symposium Chair, "At the Leading Edge of Biocatalysis," 2017 ASBMB Annual Meeting, April 22-26, 2017, Chicago, IL.

15) Vice-Chair, Gordon Research Conference on Metals in Biology, January 21-26, 2018, Ventura, CA.

16) Co-Chair, 37th Annual Penn State Summer Symposium in Molecular Biology: Frontiers in Metallobiochemistry, June 6-8, 2018, University Park, PA

17) Chair, Gordon Research Conference on Metals in Biology, January 27 – February 1, 2019, Ventura, CA.

18) Session Chair, Virtual Biocatalysis and Protein Engineering Meetup, March 18, 2021.

19) Discussion Leader, Gordon Research Conference on Metals in Biology, January 21-26, 2024, Ventura, CA.

SELECTED SERVICE ACTIVITIES

To Industry

Expert Witness for PureCircle SDN BHD and PureCircle USA Inc., in Case No. SA CV 18-01679 NS (JD Ex) PureCircle USA Inc. et al. v. SweeGen, Inc. et al.	Aug. 2020 – June 2022
Expert Witness for Dow AgroSciences, ICC Case No. 18892/VRO/AGF BAYER CROPSCIENCE, AG and BAYER CROPSCIENCE NV v. DOW AGROSCIENCES LLC, MYCOGEN PLANT SCIENCE, INC., AGRIGENETICS, INC. d/b/a MYCOGEN SEEDS, LLC, and PHYTOGEN SEED COMPANY, LLC	July 2014 – Aug. 2014
Expert Witness for Dow AgroSciences, LLC, ICC Case No. 18892/VRO/AGF/ZF Expert Witness for the Defendant, Bayer CropScience AG *v* Dow AgroSciences, LLC, C.A. No. 10-1045-RMB-JS, U.S. Dist. Ct. Del. (decided in favor of the defendant Sept. 27, 2012)	June 2011 – Sept. 2012
Member, Scientific Advisory Board, Renewable Energy Group (formerly, LS9 Incorporated), South San Francisco, CA	Dec. 2011 – 2015

To Government Agencies

Faculty Participant, NSF Workshop on Science Policy at The Pennsylvania State University	March 12, 1996
Member, Special Emphasis Panel to Review Post-Doctoral Fellowship Applications Biochemistry Initial Review Group (IRG), NIH	June 30, 2000
Member (*ad hoc*), Biochemistry IRG, NIH	June 14-15, 2001
Member, Special Emphasis Panel to Review Grant Application, Physiological Chemistry IRG, NIH	July 19, 2001

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Review of Proposals (9) for the National Science Foundation	2002-2007
Member, IRG for Post-Doctoral Fellowship Applications, NIH	March 1, 2007
Member (*ad hoc*), Macromolecular Structure and Function E IRG, NIH	June 4-5, 2007
Member (*ad hoc*), Molecular Biochemistry Review Panel, NSF	October 29-31, 2007
Permanent Member, Macromolecular Structure and Function E IRG, NIH	Oct. 2008-July 2012
Member (ad hoc), Macromolecular Structure and Function E IRG, NIH	October 17, 2012
Member, DOE ARPA-E REMOTE (Reducing Emissions using Methanotrophic Organisms for Transportation Energy) Review Panel	August 1, 2013
Co-Chair, Special Emphasis Panel to Review P01 Application, NIH	April 4, 2014
Member, Special Emphasis Panel F04A-W(20)L for fellowship review, NIH	Feb. 17-18, 2015
Member, Special Emphasis Panel to Review Applications for New "Maximizing Investigator's Research Award" (MIRA) Program, NIGMS, NIH	Nov. 9-10, 2015
Member, Mechanistic Molecular Biophysics II Review Panel, NSF	March 28-29, 2016
Member, Special Emphasis Panel 2017/05 ZRG1 BCMB-G (02) M, NIH	February 22-23, 2017
Member, Enzyme Structure and Function Review Panel for DE-FOA-0001664, DOE	March 2, 2017
Member, Special Emphasis Panel to Review Applications for "Early Stage Investigator Maximizing Investigator's Research Award" (ESI-MIRA) Program, NIGMS, NIH	March 8-9, 2018
Member, Special Emphasis Panel to Review Applications for "Early Stage Investigator Maximizing Investigator's Research Award" (ESI-MIRA) Program, NIGMS, NIH	March 14-15, 2019
Member, Special Emphasis Panel to Review K99 "Pathway to Independence" Applications, NIGMS, NIH	March 11, 2021
Member, Special Emphasis Panel to Review Applications for "Early Stage Investigator Maximizing Investigator's Research Award" (ESI-MIRA) Program, NIGMS, NIH	March 8-9, 2022

To Journals and Reference Book Series

Contributing Member, *Faculty of 1000*	2014-2016
Editorial Advisory Board Member, *Biochemistry*	2013-2015
Editorial Advisory Board Member, *Journal of Biological Chemistry*	2007-2011
Editorial Advisory Board Member, *Journal of Biological Inorganic Chemistry*	2007-2012
Special Guest Editor, *Proceedings of the National Academy of Sciences U. S. A.*	2007,8,9,11,15,19
Section Co-Editor (with V. Bandarian and S. Booker), *Comprehensive Natural Products III: Chemistry and Biology* (Volume 5 – *Enzymes and Enzyme Mechanisms: Radicals and Metalloenzymology*)	2020

To the Metallobiochemistry Community

Co-chair, Biennial Penn State Bioinorganic Workshops (visit http://sites.psu.edu/bioinorganic/)

1st Workshop - 36 trainees, 46 participants	June 1-2, 2010
2nd Workshop - 78 trainees, 123 participants	May 29 - June 6, 2012
3rd Workshop - 80 trainees, 162 participants	May 27 - June 4, 2014
4th Workshop - ~ 70 trainees, 154 participants	June 2-10, 2016
5th Workshop - ~ 80 trainees, 160 participants	June 1-5, 2018

SELECTED TRAINEES

Ph.D. Graduates

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- Dr. John C. Price, Ph.D. 2005, Assistant Professor of Chemistry and Biochemistry, Brigham Young University, 2013-2019; Associate Professor 2019-present
- Dr. Lana Saleh, Ph.D. 2005, Associate Director of Research, New England BioLabs (2004 Alumni Association Dissertation Award)
- Dr. Megan L. Matthews, Ph.D. 2011, Assistant Professor of Chemistry, University of Pennsylvania, 2017-present (2010 Alumni Association Dissertation Award; 2018 Penn State Alumni Achievement Award)
- Dr. Laura M. K. Dassama, Ph.D. 2013, Assistant Professor of Chemistry, Stanford University, 2018-present (2013 Alumni Association Dissertation Award; 2019 Penn State Alumni Achievement Award)
- Dr. Lauren Rajakovich, Ph.D. 2017, Assistant Professor of Chemistry, University of Washington, 2022-present
- Dr. Ryan Martinie, Ph. D. 2018, Assistant Professor of Chemistry, Hamilton College, 2021-present
- Dr. Noah Dunham, Ph.D. 2018, Senior Scientist, Merck Biocatalysis 2021-present
- Dr. Rachelle Copeland, Ph.D. 2021, Scientist I, Automation and Analytical Sciences Codexis, Inc. 2021-present

Post-doctoral Fellows

- Dr. Gang Xing, Senior Principal Scientist, Pfizer, 2018-present
- Dr. Yisong Guo, Associate Professor of Chemistry, Carnegie Mellon University, 2014-present
- Dr. Maria-Eirini Pandelia, Assistant Professor of Biochemistry, Brandeis University, 2016-present
- Dr. Wei-chen Chang, Associate Professor of Chemistry, North Carolina State University, 2016-present
- Dr. Alexey Silakov, Assistant Professor of Chemistry, Penn State University, 2017-present

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APPENDIX B

APPENDIX B

Exhibit	Description
1001	U.S. Patent No. 10,947,555 ("the '555 patent").
1002	U.S. Patent No. 11,149,283 ("the '283 patent").
1003	U.S. Patent No. 11,299,745 ("the '745 patent").
1004	Provisional U.S. App. No. 60/567052
1005	Certified File History for U.S. App. No. 11/587893
1006	Certified File History for U.S. App. No. 12/951813
1007	Certified File History for U.S. App. No. 14/820893
1008	Certified File History for U.S. App. No. 15/288,406
1009	Certified File History for U.S. App. No. 17/143,824
1010	Certified File History for U.S. App. No. 17/175,966
1011	Certified File History for U.S. App. No. 17/403,476
1019	J.R. Chekan et al., *Molecular Basis for Enantioselective Herbicide Degradation Imparted by Aryloxyalkanoate Dioxygenases in Transgenic Plants*, 27 PROC NAT'L ACAD. SCI. 13299-13304 (2019)
1020	J.R. Chekan et al., Supplementary Information, *Molecular Basis for Enantioselective Herbicide Degradation Imparted by Aryloxyalkanoate Dioxygenases in Transgenic Plants*, 27 PROC NAT'L ACAD. SCI. 13299-13304 (2019)
1021	U.S. Patent Pub. No. 2005/107437
1023	J.M. Elkins, R.P. Hausinger et al., *X-ray Crystal Structure of* Escherichia coli *Taurine/α- Ketoglutarate Dioxygenase Complexed to Ferrous Iron and Substrates,* 41 BIOCHEMISTRY 5185-5192 (2002)

1025	R. Hausinger, *Fe(II)/α-Ketoglutarate-Dependent Hydroxylases and Related Enzymes*, 39 CRIT. REV. BIOCHEM. MOLECULAR BIO. 21-68 (2004)
1027	D.A. Hogan, R.P. Hausinger et al., *Site-Directed Mutagenesis of 2,4-Dichlorophenoxyacetic Acid/α-Ketoglutarate Dioxygenase. Identification of Residues Involved in Metallocenter Formation and Substrate Binding*, 275 J. BIO. CHEM. 12400-12409 (2000)
1029	J.C. Dunning Hotopp & R.P. Hausinger, *Probing the 2,4-Dichlorophenoxyacetate/α-Ketoglutarate Dioxygenase Substrate Binding Site by Site- Directed Mutagenesis and Mechanism-Based Inactivation*, 41 BIOCHEMISTRY 9787-9794 (2002)
1035	T.A. Müller, R.P. Hausinger et al., *Structural Basis for the Enantiospecificities of* R- *and* S*Phenoxypropionate/α-Ketoglutarate Dioxygenases*, 15 PROTEIN SCI. 1356-1368 (2006)
1040	R.E. Saari, Substrate Specificity and Spectroscopic Properties of 2,4-Dichlorophenoxyacetic Acid/α-Ketoglutarate Dioxygenase, Doctoral Thesis, Mich. State Univ. (July 6, 1998)
1041	R.E. Saari, R.P. Hausinger, et al., *Stereospecific Degradation of the Phenoxypropionate Herbicide Dichlorprop*, 6 J. MOLECULAR CATALYSIS B:ENZYMATIC 421-428 (1999)
1042	K.M. Schleinitz et al., *Localization and Characterization of Two Novel Genes Encoding Stereospecific Dioxygenases Catalyzing 2(2,4-Dichlorophenoxy)Propionate Cleavage in* Delftia acidovorans *MC1*, 70 APPLIED & ENVTL. MICROBIO. 5357-5365 (2004)
1043	U.S. Patent No. 6,153,401
1044	W.R. Streber et al., *Analysis, Cloning, And High-Level Expression of 2,4-Dichlorophenoxyacetate Monooxygenase Gene* tfdA *of* Alcaligenes eutrophus JMP134, 169 J. BACTERIOLOGY 2950-2955 (1987)
1045	W.R. Streber & L. Willmitzer, *Transgenic Tobacco Plants Expressing a Bacterial Detoxifying Enzyme Are Resistant to 2,4-D*, 7 BIOTECHNOLOGY 811-816 (1989)

1046	E. Vedler et al., *The Completely Sequenced Plasmid pEST4011 Contains a Novel IncP1 Backbone and a Catabolic Transposon Harboring* tfd *Genes for 2,4-Dichlorophenoxyacetic Acid Degradation*, 186 J. BACTERIOLOGY 7161-7174 (2004)
1052	C.T. Larue et al., *Development of Enzymes for Robust Aryloxyphenoxypropionate and Synthetic Auxin Herbicide Tolerance Traits in Maize and Soybean Crop*, 75 PEST MGMT. SCI. 2086-2094 (2019)
1054	Dr. Hausinger's Declaration.
2001	Fumiyasu Fukumori and Robert P. Hausinger, *Purification and Characterization of 2,4-Dichlorophenoxyacetate/α-Ketoglutarate Dioxygenase*, J. of Biol. Chem., Vol. 268, No. 32, pp. 24311-17 (1993) ("Fukumori").
2002	Sealed Version of Defendants' Counterclaims to Plaintiff's Second Amended Complaint, *Corteva Agriscience LLC v. Monsanto Co.*, No. 22-cv-1046-GBW (D. Del.), D.I. 143 (excerpted pages 38 to 65 and accompanying exhibits) (CONFIDENTIAL – FILED UNDER SEAL).
2003	Redacted Version of Defendants' Counterclaims to Plaintiff's Second Amended Complaint, *Corteva Agriscience LLC v. Monsanto Co.*, No. 22-cv-1046-GBW (D. Del.), D.I. 143 (excerpted pages 38 to 65 and accompanying exhibits).
2004	Protein sequences (CONFIDENTIAL – FILED UNDER SEAL).
2005	Protein sequences (CONFIDENTIAL – FILED UNDER SEAL).
2006	Protein presentation (CONFIDENTIAL – FILED UNDER SEAL).
2007	Protein presentation (CONFIDENTIAL – FILED UNDER SEAL).
2008	Protein sequences (CONFIDENTIAL – FILED UNDER SEAL).
2009	Protein assay analysis (CONFIDENTIAL – FILED UNDER SEAL).
2010	Protein presentation (CONFIDENTIAL – FILED UNDER SEAL).

2011	Protein sequences and correspondence (CONFIDENTIAL – FILED UNDER SEAL).
2012	GenBank Accession No. MH043112.
2013	GenBank Accession No. MH043114.
2014	GenBank Accession No. MH043115.
2016	Transcript from Deposition of Dr. Hausinger (Apr. 4, 2024).
2018	Robinson, *Enzymes: principles and biotechnological applications*, 59 ESSAYS BIOCHEM. 1–41 (2015) ("Robinson").
2019	Tian and Skolnick, *How Well is Enzyme Function Conserved as a Function of Pairwise Sequence Identity?* 333 J. MOL. BIOL. 863–882 (2003) ("Tian").
2020	Tipton and Boyce, *History of the enzyme nomenclature system*, 16(1) BIOINFORMATICS 34–40 (2000) ("Tipton").
2021	Rost, *Twilight zone of protein sequence alignments*, 12(2) PROTEIN ENGINEERING 85–94 (1999) ("Rost 1999").
2023	Chung and Subbiah, *A structural explanation for the twilight zone of protein sequence homology*, 4(10) STRUCTURE 1123–27 (1996) ("Chung").
2024	Wilson et al., *Assessing Annotation Transfer for Genomics: Quantifying the Relations between Protein Sequence, Structure and Function through Traditional and Probabilistic Scores*, 297 J. MOL. BIOL. 233–249 (2000) ("Wilson").
2025	Minshull et al., *Predicting enzyme function from protein sequence*, 9(2) CURR. OPIN. CHEM. BIOL. 202-09 (2005) ("Minshull").
2026	Devos and Valencia, *Practical Limits of Function Prediction*, 41 PROTEINS 98–107 (2000) ("Devos").
2027	Todd et al., *Evolution of Function in Protein Superfamilies, from a Structural Perspective*, 307 J. MOL. BIOL. 1113-43 (2001) ("Todd").

2028	Rost, *Enzyme Function Less Conserved than Anticipated*, 318 J. MOL. BIOL. 595–608 (2002) ("Rost 2002").
2029	desJardins et al., *Prediction of Enzyme Classification from Protein Sequence without the use of Sequence Similarity*, 5 PROC. INT. CONF. INTELL. SYST. MOL. BIOL. 92–99 (1997) ("desJardins").
2030	Price and Arkin, *PaperBLAST: Text Mining Papers for Information about Homologs*, 2(4) MSYSTEMS e00039 1–10 (2017) ("Price").
2032	Galperin et al., *Analogous Enzymes: Independent Inventions in Enzyme Evolution*, 8 GENOME RESEARCH 779–90 (1998) ("Galperin").
2033	Dym and Eisenberg, *Sequence-structure analysis of FAD-containing proteins*, 10 PROTEIN SCIENCE 1712–28 (2001) ("Dym").
2034	Minarik et al., *Malate Dehydrogenases – Structure and Function*, 21 GEN. PHYSIOL. BIOPHYS. 257–65 (2002) ("Minarik").
2037	Wedege and Frøholm, *Human Antibody Response to a Group B Serotype 2a Meningococcal Vaccine Determined by Immunoblotting*, 51(2) Infection and Immunity 571–78 (1986) ("Wedege").
2038	Chica et al., *Semi-rational approaches to engineering enzyme activity: combining the benefits of directed evolution and rational design*, 16(4) CURR. OPIN. BIOTECHNOL. 378–84 (2005) ("Chica").
2040	Altschul et al., *Gapped BLAST and PSI-BLAST: a new generation of protein database search programs*, 25(17) NUCLEIC ACIDS RESEARCH 3389–3402 (1997) ("Altschul").
2041	Müller and Vingron, *Modeling Amino Acid Replacement*, 7(6) JOURNAL OF COMPUTATIONAL BIOLOGY 761–76 (2000) ("Müller").
2044	Ng and Henikoff, *SIFT: predicting amino acid changes that affect protein function*, 31(13) NUCLEIC ACIDS RESEARCH 3812–14 (2003) ("Ng").
2047	Llewelyn et al., *Monoclonal Antibodies in Medicine*, 305 BMJ 1269–72 (1992) ("Llewelyn").
2048	GenBank Accession No. MH043108.

2049	GenBank Accession No. MH043109.
2050	GenBank Accession No. MH043110.
2052	Last and Llewellyn, *A detoxification gene in transgenic Nicotiana tabacum confers 2,4-D tolerance*, 47 WEED SCIENCE 401–04 (1999) ("Last and Llewellyn").
2053	Nakajima et al., *Crystal structures of two tropinone reductases: Different reaction stereospecificities in the same protein fold*, 95 PROC. NATL. ACAD. SCI. USA 4876–81 (1998) ("Nakajima").
2055	Llewellyn and Last, *Genetic Engineering of Crops for Tolerance to 2,4-D*, HERBICIDE-RESISTANT CROPS 159–74 (1996) ("Duke").
2056	Vencill et al., *Herbicide Handbook 8th Edition*, Weed Science Society of America 111–115, 133–135, 281–283, 453–454 (2002) ("Herbicide Handbook").
2057	Dayhoff et al., *A Model of Evolutionary Change in Proteins*, 5(3) ATLAS OF PROTEIN SEQUENCE AND STRUCTURE 345–52 (1978) ("Dayhoff").
2061	Sequence alignment of sequences from Exs. 2012-2014 and 2048-2050.