UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

BAYER CROPSCIENCE LP, MONSANTO COMPANY, AND INARI AGRICULTURE, INC.[1]

Petitioners,

v.

CORTEVA AGRISCIENCE LLC,

Patent Owner.

Case No. IPR2023-01036
U.S. Patent No. 10,947,555

PETITIONERS' DEMONSTRATIVE EXHIBITS

[1] The Board joined Inari Agriculture, Inc. as a party (Petitioner) to this proceeding via a Motion for Joinder in IPR2024-00179.

Pursuant to the Board's Order Setting Oral Argument dated August 29, 2024 (Paper 36), Petitioners Bayer CropScience LP and Monsanto Co.'s demonstrative exhibits are appended to this filing.

Dated: September 19, 2024

Respectfully submitted,

/Ben Picozzi/
Ben Picozzi
Reg. No. 76,862
Backup Counsel for Petitioners

Monsanto Company & Bayer CropScience LP v. Corteva Agriscience LLC

IPR2023-01036 – U.S. Patent No. 10,947,555
IPR2023-01037 – U.S. Patent No. 11,149,283
IPR2023-01038 – U.S. Patent No. 11,299,745

September 24, 2024

Grounds for the Three Proceedings

Ground IA	**Anticipation** October 7, 2016 Priority Date
Ground IB	**Obviousness** October 7, 2016 Priority Date
Grounds IIA-IIB	**Obviousness** April 30, 2004 Priority Date

’555 Patent Claims 1 & 2

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.

2. The plant cell of claim **1** wherein said AAD-1 motif has the general formula of:

$HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R(X)_{282\text{-}284}R$, wherein $(X)_{282\text{-}284}$ represents a sequence of 3 amino acids.

EX1001, claims 1-2; *see also* EX1002, claims 1-2; EX1003, claims 1-2

No Support for the Claimed AAD-1 Motif

Priority Applications 2004/2005

The subject invention relates in part to the identification of an enzyme that is not only able to degrade 2,4-D, but also surprisingly possesses novel properties, which distinguish the enzyme of the subject invention from previously known *tfdA* proteins, for example. Even though this enzyme has very low homology to *tfdA*, the genes of the subject invention can still be generally classified in the same overall family of a-ketoglutarate-dependent dioxygenases. This family of proteins is characterized by three conserved histidine residues in 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.

Claim Added in October 2016 by Preliminary Amendment

ii) an AAD-1 motif having the general formula of: $HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$ . . .

Specification Amended in April 2019

Mapping the α-ketoglutarate-dependent dioxygenase motif disclosed in the previous paragraph to the sequence of SEQ ID NO: 9 reveals the AAD-1 motif of $HX_{112}D(X)_{114\text{-}137}T(X)_{139\text{-}269}H(X)_{271\text{-}280}R$ . . .

EX1004, [0059]; EX1005, [0085]; EX1008, -003, -005-009, -480; 1036 Pet. 11-13 , 27-28;
1037 Pet. 11-13, 27-28; 1038 Pet. 11-13, 28-29; 1036 ID 27, 30-31; 1037 ID 28, 31-32; 1038 ID 29, 32-33

Institution Decision

Instead, we find persuasive at this stage Dr. Hausinger's testimony that such a mapping would only confirm what was already taught in the art; that is, the known RdpA (SEQ ID NO: 9) enzyme shares canonical active site residues characteristic of αKGD dioxygenases.

We also question whether a "mapping" that purports to describe something new and unique about a structure-function relationship defined by an AAD-1 motif raises potential obviousness issues that fall short of what is needed.

Rivera: Prior Art Cannot Supply Missing Claim Limitations

Rivera v. ITC, 857 F.3d 1315, 1322-23 (Fed. Cir. 2017)

"The knowledge of ordinary artisans may be used to inform what is actually in the specification, *see Lockwood*, 107 F.3d at 1571, but not to teach limitations that are not in the specification, even if those limitations would be rendered obvious by the disclosure in the specification. *Id.* at 1571–72."

Filing Dates of Provisional and PCT Applications

April 30, 2004
Provisional Application

May 2, 2005
PCT Application

2004 | **2005**

EX1004, 3; EX1005, 1; EX1042, 1; 1036, 1037, 1038 POR 3, 42, 48-49; 1036, 1037, 1038 Sur-reply 15

Corteva: Schleinitz (EX1042) Is "Prior Art"

April 30, 2004
Provisional Application

September 2004
Schleinitz Published

May 2, 2005
PCT Application

2004 | 2005

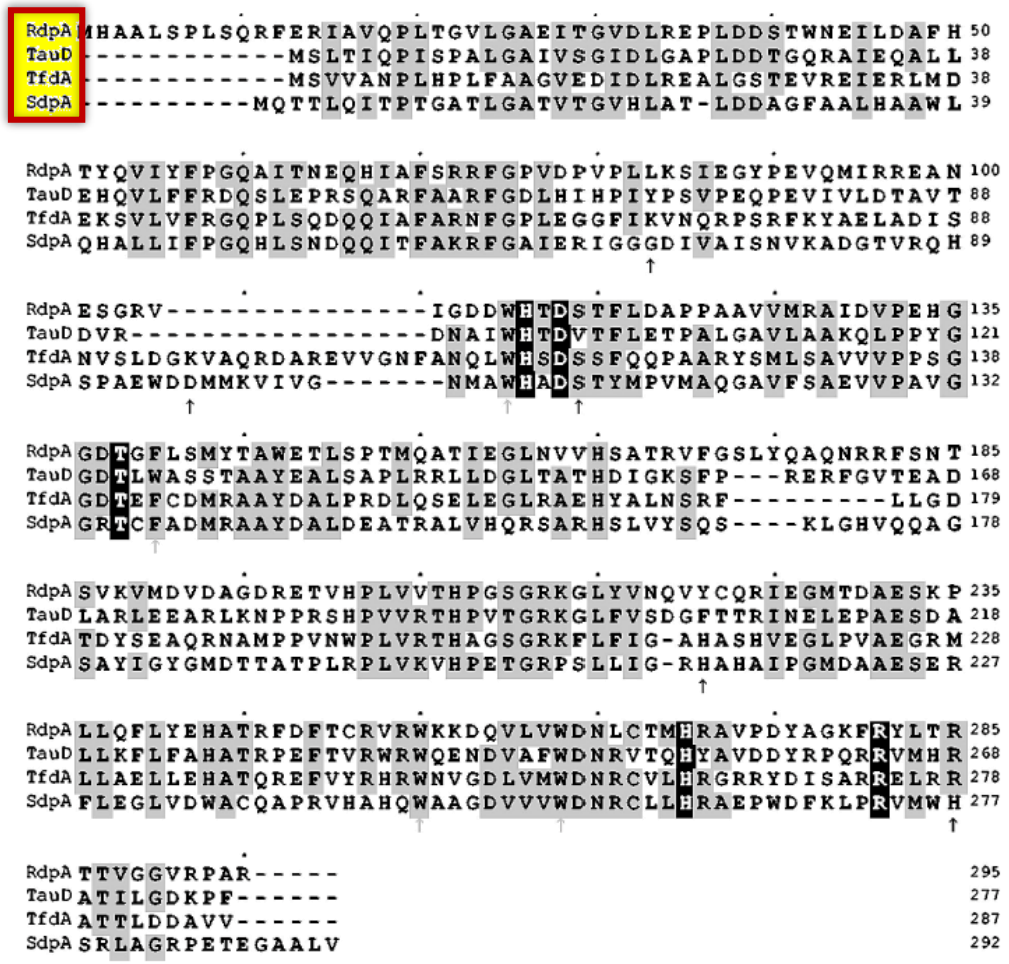
EX1004, 3; EX1005, 1; EX1042, 1; 1036, 1037, 1038 POR 3, 42, 48-49; 1036, 1037, 1038 Sur-reply 15

Schleinitz Does Not Describe the Claimed AAD-1 Motif

The functionally important amino acid sequence motif HX(D/E)$X_{23\text{-}26}$(T/S)$X_{114\text{-}183}$H$X_{10\text{-}13}$R/K, which is highly conserved in group II-ketoglutarate-dependent dioxygenases, was present in both dichlorprop-cleaving enzymes.

The presence of the functionally essential motif HX(D/E)$X_{23\text{-}26}$(T/S)$X_{114\text{-}183}$H$X_{10\text{-}13}$R/K indicates that TfdA is related to several other (referred to as group II) αKG-dependent dioxygenases (18).

The functionally important motif HX(D/E)$X_{23\text{-}26}$(T/S)$X_{114\text{-}183}$H$X_{10\text{-}13}$R/K, which is conserved throughout group II αKG-dependent dioxygenases (18), is present in both RdpA and SdpA.



AAD-1 Motif: Anticipation

'555 Patent Petition

9-10. Although Corteva '437 does not support the '555 patent's broad genus claims, *supra* Section VII.A, it discloses transgenic plants and plant cells within their scope. It therefore anticipates claims 1-34 because "a later genus claim limitation is anticipated by, and therefore not patentably distinct from, an earlier species claim." *Eli Lilly*, 251 F.3d at 971. EX1054, ¶¶ 111-207.

Corteva '437 also discloses and claims proteins having the claim 1 AAD-1 motif. The applicant represented during prosecution that SEQ ID NO: 9-10 share the claimed AAD-1 motif. EX1008, COR-AAD1-00000487-489 ("This disclosed AAD-1 motif is embodied in the disclosed AAD-1 sequences of SEQ ID Nos: 9, 10 . . . "), COR-AAD1-00000679. SEQ ID NO: 9-10 have a histidine (H) at position 111, aspartic acid (D) at position 113, threonine (T) at position 138, histidine (H) at position 270, and arginine (R) at position 281. EX1021, [0051]-[0052], SEQ ID NO: 9-10; EX1054, ¶ 122.

1036 Pet. 17 n.1, 19, 41, 44; *see also* 1037 Pet. 17 n.1, 19-20, 41, 44; 1038 Pet. 17-18 n.1, 19-20, 41, 44-45

No Support for the Claimed Activity

'555 Patent	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:
Institution Decision	59. At present, it is not clear where, if at all, the priority applications include sufficient disclosures from which the POSA would understand a structure-function correlation between the AAD-1 motif and (*R*)-FOP degradation.

EX1001, claim 1; 1036 ID 46; 1037 ID 47; 1038 ID 48; *see also* EX1002, claim 1; EX1003, claim 1

Unknown Structure-Function Relationship Insufficient

PureCircle USA Inc. v. SweeGen, Inc.,
No. 2022-1946, 2024 WL 20567, at *4 n.10 (Fed. Cir. Jan. 2, 2024)

In *Ajinomoto* "there was a 'well-known link between consensus sequence and promoter strength' as 'promoters having fewer departures from a 'consensus sequence' in a promoter are generally stronger' and 'the genus of more potent promoters was already well explored in the relevant art.' *Id*. at 1359-60. Even though there was some evidence that 'deviations from [the consensus] sequence d[id] not always decrease promoter strength,' this Court held that '[a]dequate written description does not require a perfect correspondence between the members of the genus and the asserted common structural feature.' *Id*. at 1360. Thus, *Ajinomoto* stands for the idea that where there are structural features common to a genus, the structure-function correlation does not need to be perfect and some testing—appropriate to the knowledge of a POSA—is allowed, not that an unknown structure-function correlation along with extensive testing can satisfy written description."

How the Specification Distinguishes the Patent

tfdA proteins, for example. More specifically, the subject invention relates to the use of an enzyme that is capable of degrading both 2,4-D and AOPP herbicides, in an enantio-specific manner. No α-ketoglutarate-dependent dioxygenase enzyme has previously been reported to have the ability to degrade herbicides of different chemical classes and modes of action. The preferred enzyme and gene for use according

enon), misapplication, vandalism and the like. An additional benefit of the AAD-1 gene is that unlike all tfdA homologues characterized to date, AAD-1 is able to degrade the R-enantiomers (herbicidally active isomers) of the chiral phenoxy auxins (e.g., dichlorprop and mecoprop) in addition to achiral phenoxy auxins (e.g., 2,4-D, MCPA, 4-chlorophenoxyacetic acid). See Table 1. Multiple mixes of different

EX1004, [0017], [0051]; EX1005, [0018], [0074]; EX1001, 4:32-46, 8:10-17; EX1002, 4:45-59, 8:17-23; EX1003, 4:48-62, 8:19-25; 1036, 1037, 1038 Reply 1; 1036, 1037, 1038 ID 8

Chekan, Published 2019

Chekan 2019

Molecular basis for enantioselective herbicide degradation imparted by aryloxyalkanoate dioxygenases in transgenic plants

Jonathan R. Chekan[a,b,1], Chayanid Ongpipattanakul[a,1], Terry R. Wright[c], Bo Zhang[d], J. Martin Bollinger Jr.[d,e], Lauren J. Rajakovich[e], Carsten Krebs[d,e], Robert M. Cicchillo[c], and Satish K. Nair[a,b,f,2]

Despite the agronomic importance of AAD enzymes in transgenic crops, little is known about protein–substrate interactions or how these enzymes catalyze enantioselective oxidation of herbicides. To date, no crystal structure has been reported for any member of the AAD family, and there is, therefore, an insufficient knowledge base to distinguish bona fide AADs from other enzymes of the Fe(II)/αKG dioxygenase class. In this study, we used a genomic context-

Institution Decision Discussing Chekan

Also, the 2019 publication echoes a long-standing recognition that, as with other enzymes in the αKGD family, the structural basis for stereoselectivity of the AAD enzymes was not well understood. Ex. 1019, 13300 ("[L]ittle is known about protein-substrate interactions or how these [AAD] enzymes catalyze enantioselective oxidation of herbicides.");

Chekan (2019) and (R)-FOPs

Genes involved in the microbial degradation pathway for 2,4-D were identified nearly 4 decades ago, but insights into the structure-function relationships within the aryloxyalkanoate dioxygenase family have been lacking.

The cocrystal structures with (*R*)-diclofop and (*R*)-cyhalofop provide the basis for understanding the substrate tolerance of AAD-1 against the AOPP class of grass-specific herbicides. AOPPs have scaffolds that are similar to that of phenoxycarboxylic synthetic auxins but contain an additional aromatic ring attached to the phenoxy moiety. Both (*R*)-diclofop and (*R*)-cyhalofop bind at the AAD-1 active site in a manner similar to that of the phenoxycarboxylic synthetic auxin, with their chiral carbon oriented directly above the metal and the methyl group projecting into the aforementioned hydrophobic pocket (Fig. 4 *A* and *B*). The additional aryl rings of both (*R*)-diclofop and (*R*)-cyhalofop are accommodated within a solvent-exposed tunnel comprising Argl04, Aspl08, Aspl09, Val170, Phel82, and Val220. There are limited interactions between the extended ring of these AOPP herbicides and residues in AAD-1, and only Val220 makes van der Waals contacts with the ring. A superposition of these structures with that of AAD-1 bound to the smaller (*R*)-dichlorprop reveals that enzyme residues Argl04, Asp109, and Gln219 shift away from the tunnel to accommodate the second ring. The presence of this solvent-exposed tunnel was not evident in earlier modeling studies (50). The lack of significant interactions between residues in the tunnel and the AOPP herbicides suggests that AAD-1 also may be able to degrade other molecules based on the (*R*)-dichlorprop scaffold (with an aryloxyalkanoate moiety) (5, 41).

Schleinitz, Published 2004

Schleinitz	The basis of the enantiospecificity of the dichlorprop-cleaving enzymes is not known. It is known that the tertiary structure of homologous proteins showing at least 20% identity of amino acid sequence is conserved (41). Among group II αKG dioxygenases a high degree of structural conservation was observed between clavaminate synthase and TauD, which share as little as 7.7% primary sequence identity (10). It is therefore likely that 2,4-D and dichlorprop-cleaving enzymes are structurally very similar and that substrate specificity is determined by only a few amino acids. Site-specific mutagenesis studies are needed to reveal the molecular basis of the substrate specificity and stereospecificity of RdpA and SdpA.
Institution Decision Discussing Schleinitz	And, even in that context, Schleinitz reported that "[t]he basis of the enantiospecificity of the dichlorprop-cleaving enzymes is not known." Id. at 5362–63 (disclosing it "likely . . . that substrate specificity is determined by only a few amino acids" and "[s]ite-specific mutagenesis studies are needed to reveal the molecular basis of the substrate specificity and stereospecificity of RdpA and SdpA"). If anything, this highlights a lack of understanding and the unpredictability about (R)- and (S)- enantiomer substrate specificity among the family of αKGD enzymes in the 2004/2005 timeframe.

EX1042, 5362-63; 1036 Reply 3, 6; 1037 Reply 3, 6; 1038 Reply 3, 6; 1036 ID 46; 1037 ID 47; 1038 ID 49

Müller, Published 2006

Müller	In contrast to these conserved residues, essentially nothing is known about the phenoxypropanoic acid-binding sites of these proteins, especially with regard to the structural basis of enantiospecificity.
Bollinger Discussing Müller	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.

EX1035, 1358; EX2015 ¶ 153; 1036, 1037, 1038 Pet. 31; 1036, 1037, 1038 POR 62-63; 1036 ID 49-50; 1037 ID 51; 1038 ID 52

Achiral Phenoxy Auxins (e.g., 2,4-D)

'555 Patent	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:
Petition	The '555 patent uses the term "aryloxyalkanoate" to refer collectively to achiral phenoxy auxins (*e.g.*, 2,4-D), chiral phenoxy auxins (*e.g.*, (*R*)-mecoprop), and chiral aryloxyphenoxypropionates (*e.g.*, (*R*)-diclofop). EX1001, 3:49-56, claims 6-10; EX1054, ¶ 44.
Hausinger Opening Declaration	44. The '555 patent uses the term "aryloxyalkanoate" to refer collectively to a group of herbicides including the achiral phenoxy auxins, such as 2,4-D; the chiral phenoxy auxins, such as dichlorprop and mecoprop; and chiral aryloxyphenoxypropionates, such as diclofop. EX1001 ('555 Pat.), 3:49-56

EX1001, claim 1; 1036, 1037, 1038 Pet. 8; EX1054, ¶ 44

AAD-1 Activity

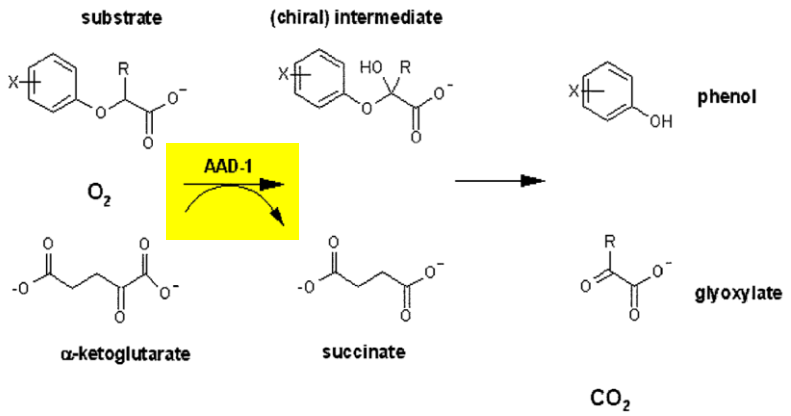
Corteva Sur-reply	But even if a similar effect were reported for RdpA variants, Petitioners fail to explain how the ability to additionally degrade a small amount of an (S)-enantiomer would take such variants outside the scope of the claims. *See* Ex. 1001, 52:35-55:34, Table 4 (reporting AAD-1 activity on (S)-cyhalofop); Ex. 2066, 31:7-15.
'555 Patent	The results are shown in FIG. 6B. The activity of the enzyme on (R)-haloxyfop was equivalent to that on (R,S)-haloxyfop whereas no activity could be seen on the S-enantiomer of cyhalofop indicating that AAD-1 (vl) has R specificity on AOPPs.
	Table 33 shows data from additional substrates tested with AAD-1 (v1) and AAD-2 (v1) [including (S)-cyhalofop] that confirm that AAD-2 (v1) is specific for (S)-enantiomer substrates, in contrast to AAD-1 (v1) which is specific for (R)-enantiomers.

1036, 1037, 1038 Sur-reply 7; 1036, 1037, 1038 Pet. 26; 1036, 1037, 1038 ID 16 n.8; 1036, 1037, 0138 POR 22, 70; EX1001, 52:29-33, 112:58-61; *see also* EX1002, 52:3-7, 110:37-40; EX1003, 52:2-6, 110:56-59

’555 Patent and File History

’555 Patent

tfdA proteins, for example. More specifically, the subject invention relates to the use of an enzyme that is capable of degrading both 2,4-D and AOPP herbicides, in an enantio-specific manner. No α-ketoglutarate-dependent dioxygenase enzyme has previously been reported to have the ability to degrade herbicides of different chemical classes and modes of action. The preferred enzyme and gene for use according



- Addition of O_2 is *stereospecific*
- Breakdown of intermediate to phenol + glyoxylate is spontaneous

FIG. **1** shows a general scheme for dioxygenase cleavage of phenoxy auxin or AOPP herbicides.

Nair Declaration

“Comparison of the sequence alignments of AAD-1 (an (R)-specific enantiomer enzyme) and (S)-specific enantiomer dioxygenases (including AAD-2, TfdA, and another homologue, AAD-12) confirmed a divergence in the length and identity of residues which mapped to the substrate-capping loop as identified by comparison of substrate-bound crystal structures. Based on the disclosure contained in the ’406 Application, those skilled in the art would have compared and recognized the importance of the sequence differences between AAD-1 variants [(R)specific dioxygenase] having dual herbicide MOA Fe/α-KG dioxygenase activity and (S)specific dioxygenases not having dual herbicide MOA Fe/α-KG dioxygenase activity even without crystallography information.” (brackets in original)

EX1001, 4:36-39, 5:16-17, Fig. 1; EX1008, -594-595; 1036, 1037, 1038 Pet. 13-14, 37; 1036,1037, 1038 ID 8, 17 n.8;
1036, 1037, 1038 POR 19-20; 1036, 1037, 1038 Reply 1; see EX1002, 4:49-52, 5:30-31, Fig. 1; EX1003, 4:52-55, 5:32-33, Fig. 1

No Support for the Claimed 85% Identity

Hausinger Opening Declaration

- "20^{44}" is a "rough lower estimate of possible amino acid sequences"
- "The specification discloses only three examples of proteins (AAD-1 v1, v2, and v3) with the required activity, and their amino acid sequences are nearly identical to one another."
- "The POSA could not have known, and would not have been able to predict, based on the limited information in the patent, whether any given amino acid sequence possessing the required motif and percent identity would have had the claimed activity."

EX1054, ¶¶ 76, 79, 83, 91; 1036 Pet. 30, 38-39; 1037 Pet. 30, 38-39; 1038 Pet. 30-31, 38-39

Institution Decision

Inasmuch as Patent Owner is suggesting that the disclosure announces a more universal principle for all the proteins including not just TfdA, but RdpA too—that is, if at least 85% amino acid identity, the POSA would expect the same properties—this would benefit from further evidentiary development at trial. In this vein, the parties should consider addressing evidence here about TfdA homologues sharing higher levels of identity that appear to have *different* properties. *See* Ex. 1041, 421, 427 (describing a TfdA-like protein from *A. denitrificans* with 86% and 95% amino acid identity to two other TfdA proteins but exhibiting opposite stereoselective degradation activities). *Capon*, 418 F.3d at 1360 ("The predictability or

Corteva's Response

Thus, by 2004-2005, skilled persons routinely predicted enzyme function based on sequence similarity to enzymes whose function had been experimentally determined. Ex. 2032, 779-80; Ex. 2033, 1712; Ex. 2034, 258. The studies detailed above show that this was a universal principle applied within the field of enzymology. *See, e.g.*, Ex. 2015, ¶ 36; Ex. 2026, 98; Ex. 2019, 864 ("[S]equence

Boston Scientific: Cannot Rely on "Well Known" Information If Contradicted by the Specification

Boston Scientific Corp. v. Johnson & Johnson, 647 F.3d 1353, 1366 (Fed. Cir. 2011)

"However, when the four corners of the specification directly contradict information that the patentee alleges is 'well-known' to a person of skill at the effective filing date, no reasonable jury could conclude that the patentee possessed the invention."

Specification: Type of Substitution Impacts Activity

Various properties and three-dimensional features of the protein can also be changed without adversely affecting the activity/functionality of the protein. Conservative amino acid substitutions can be tolerated/made to not adversely affect the activity and/or three-dimensional configuration of the molecule. Amino acids can be placed in the following classes: non-polar, uncharged polar, basic, and acidic. Conservative substitutions whereby an amino acid of one class is replaced with another amino acid of the same type fall within the scope of the subject invention so long as the substitution is not adverse to the biological activity of the compound. Table 5 provides a listing of examples of amino acids belonging to each class.

In some instances, non-conservative substitutions can also be made. However, preferred substitutions do not significantly detract from the functional/biological activity of the protein.

EX1001, 38:6-31; EX1002, 37:54-38:12; EX1003, 38:3-28; 1036, 1037, 1038 Reply 13-14

Example 1:
Activity on Achiral 2,4-D, Percentage Identity Relative to tfdA

Method for Identifying Genes that Impart Resistance to 2,4-D in Planta

As a way to identify genes which possess herbicide degrading activities in planta, it is possible to mine current public databases such as NCBI (National Center for Biotechnology Information). To begin the process, it is necessary to have a functional gene sequence already identified that encodes a protein with the desired characteristics (i.e., α-ketoglutarate dioxygenase activity). This protein sequence is then used as the input for the BLAST (Basic Local Alignment Search Tool) (Altschul et al., 1997) algorithm to compare against available NCBI protein sequences deposited. Using default settings, this search returns upwards of 100 homologous protein sequences at varying levels. These range from highly identical (85-98%) to very low identity (23-32%) at the amino acid level. Traditionally only sequences with high homology would be expected to retain similar properties to the input sequence. In this case we chose only those sequences with ≤50% homology. We go on to exemplify that cloning and recombinantly expressing homologues with as little as 27% amino acid conservation can be used to impart commercial levels of resistance not only to the intended herbicide, but also to substrates never previously tested with these enzymes.

PCR and Cloning of Gene into pET.

A single gene (rdpA) was identified from the NCBI database (see the ncbi.nlm.nih.gov website; accession #AF516752) as a homologue with only 28% amino acid identity to tfdA from *Ralstonia eutropha*. Percent identity was determined by first translating both the rdpA and tfdA DNA sequences deposited in the database to proteins, then using ClustalW in the VectorNTI software package to perform the multiple sequence alignment.

Saari 1999

denitrificans were shown to catabolize (R)-dichlorprop. Although the A. denitrificans activity exhibited stereospecificity opposite to that of the JMP134 and RASC strains, its cofactor requirements were found to be characteristic of an α-KG-dependent dioxygenase. A PCR amplification product from the DNA of this strain was shown to encode an amino acid sequence that was 95% and 86% identical to the corresponding region of TfdA in RASC and JMP134, respectively. Thus, closely related herbicide-degrading gene products appear to be capable of exhibiting opposite stereochemical degradative capabilities.

(0.1 mM) and the standard reaction components. Dichlorprop was quantitated by using chiral HPLC and 2,4-DCP was quantitated colorimetrically. Data for the (S)-dichlorprop were fit to a straight

3). The A. denitrificans activity was dependent on α-KG and ferrous ion, and was rapidly lost (in 2 min) in the absence of ascorbic acid. These cofactor requirements are characteristic of α-KG-dependent dioxygenases.

No Connection Between Tian and the Specification

activity of the exemplified proteins. Equivalent proteins will have amino acid similarity (and/or homology) with an exemplified protein. The amino acid identity will typically be at least 60%, preferably at least 75%, more preferably at least 80%, even more preferably at least 90%, and can be at least 95%. Preferred proteins of the subject invention can also be defined in terms of more particular identity and/or similarity ranges. For example, the identity and/or similarity can be 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, or 99% as compared to a sequence exemplified or suggested herein. Any number listed above can be used to define the upper and lower limits.

EX1001, 37:42-55; EX1002, 37:22-35; EX1003, 37:30-42; 1036, 1037, 1038 Pet. 12, 39; 1036, 1037, 1038 Reply 14

Tian and Related References: Naturally Occurring Proteins

TIAN EX2019, 876

"Our results suggest that for function annotation on genome sequences, . . . to transfer all four digits of an EC number, above 60% sequence identity is needed to have above 90% accuracy."

ROST EX2028, 605-06

"If we want to estimate the accuracy in transferring function in the context of genome annotation, we have to base our analysis on data sets that have a redundancy similar to that of entire proteomes."

DEVOS EX2026, 106

"In addition to the practical implications for function annotation, the results presented here can be interpreted in terms of protein evolution."

WILSON EX2024, 245

"The sequence-structure and sequence-function relationships described here provide practical information for genome annotation in terms of folds and functions."

TODD EX2027, 113

Exploring "the mechanisms for generating functional diversity during evolution," and "[t]he implications of functional diversity within superfamilies for the structural genomics project."

EX1070, ¶¶ 37, 41, 45; 1036 Reply 15-16; 1037 Reply 15-16; 1038 Reply 15-16

SWISS-PROT

TIAN EX2019, 865

"Instead of trying to define functional similarities, we directly use the functional annotation in the SWISSPROT database . . . ."

BAIROCH EX1074, 53

"Another category of data which will not be included in SWISS-PROT are synthetic sequences."

Dr. Bollinger's Declaration Regarding Enzymes and Evolution

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

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).

EX2015, ¶ 42; 1036 Reply 16; 1037 Reply 16; 1038 Reply 16

Minshull, Published in 2005

Prediction of protein function from sequence and structure is a difficult problem, because homologous proteins often have different functions. Despite this difficulty, the

The sequences of orthologous proteins are thus a potentially rich source of information regarding sequence changes that are acceptable without drastically disrupting protein function, while paralogs may provide additional information about protein sequence elements responsible for functional change.

Minshull, Published in 2005

Corteva's Sur-Reply

Minshull (Ex. 2025)
Concluding that "the use of sequence-activity relationships *should free protein engineers from the expenses and inaccuracies of high-throughput screens* . . . ." Ex. 2025, 207.

Minshull

those changes in a powerfully predictive way. In particular, the use of sequence–activity relationships should free protein engineers from the expenses and inaccuracies of high-throughput screens, and allow them to directly measure the protein properties that they really care about.

EX2025, 207; 1036 Sur-Reply 19; 1037 Sur-Reply 19; 1038 Sur-Reply 19

[redacted]

Sequences

	Seq 10		[redacted]
	Seq 11		
	Seq 12		

[redacted]

Seq ID	[redacted]
Seq10	
Seq11	
Seq12	

[redacted]

Seq ID	[redacted]
Seq10	
Seq11	
Seq12	

No Support for $X_{282\text{-}284}R$ (R285)

'555 Patent	This family of proteins is characterized by three conserved histidine residues in 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.
EX2004: [redacted]	[redacted]

EX1001, 18:2-5; EX2004, -580; 1036, 1037, 1038 Pet. 34;
1036, 1037, 1038 Reply 23-24; *see* EX1002, 18:44-48; EX1003, 18:46-49 [redacted]

R285 "Critical" for the Claimed Activity

Interview Summary

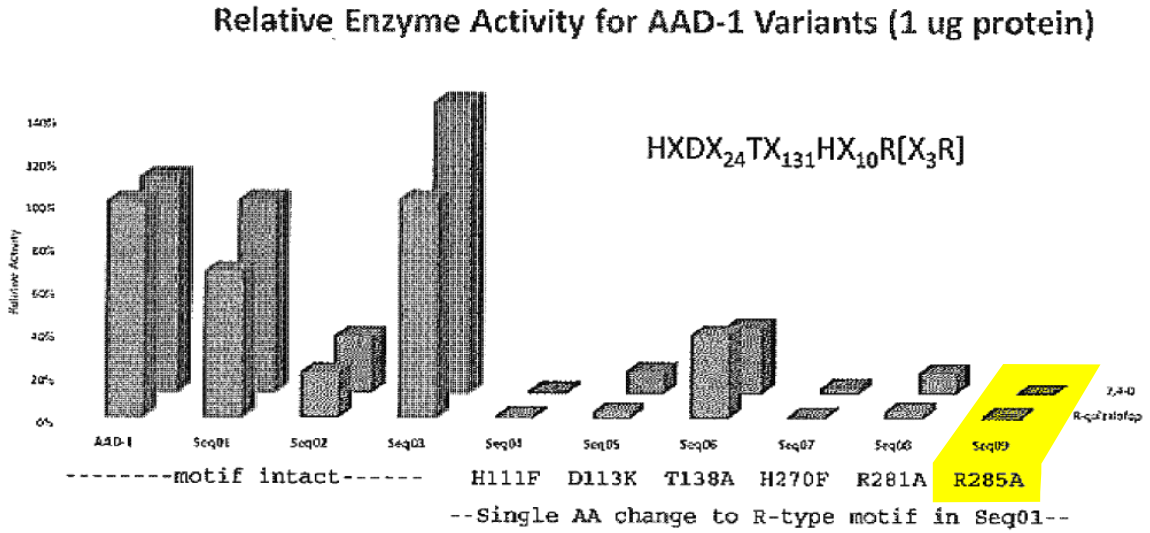
The correlation between the AAD-1 motif recited in the claims and the general motif recited in paragraph 0085 on page 17 of the Specification was discussed. The evidence in the Attachment was discussed, specifically that Applicant has modified that required amino acids in the AAD-1 motif, demonstrating that these amino acids are critical for maintaining the claimed function (see claim 15, lines 3 and 4). Applicant pointed out that the motif found in AAD-2 differs from the AAD-1 motif and does not have the claimed function. The Examiner suggested pointing out the correlation between the motif described on page 17 of the Specification and the recited AAD-1 motif found in instant SEQ ID NO: 9. No agreement was reached concerning the "at least 85% sequence identity" limitation in claim 15..

Corteva Data Submitted During Prosecution

Seq01 - Seq03 = 95% Identity (15 aa changes) to AAD-1 (SEQ ID NO. 9, motif intact)

Remaining Seq's single aa change to motif residues

H111F	Seq04
D113K	Seq05
T138A	Seq06
H270F	Seq07
R281A	Seq08
R285A	Seq09



For Discussion Purposes Only

Schleinitz: Black Arrows Indicate TfdA Binding to 2,4-D

```
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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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.

Grounds IIA-IIB: Motivation

Saari 1999	which are recycled. As an alternate illustration, the genes encoding an enzyme specific for the R isomer could be used to develop genetically engineered crops that are resistant to the herbicide of interest. Genetic engineering has already
Saari 1998	2,4-dichlorophenol. A potential application for *A. denitrificans* or the *tfdA*-like gene it contains would be protection of crops from mecoprop and dichlorprop. Cotton shows
	1997). For protection against the chiral phenoxypropionate herbicides, the pJP4-derived gene product would be ineffective (because it would not degrade the herbicidal R-enantiomer), but the gene from *A. denitrificans* could be used since its gene product degrades the R-enantiomer.

EX1040, 93; EX1041, 422; 1036 Pet. 55; 1037 Pet. 54-55; 1038 Pet. 56-57

Grounds IIA-IIB: Expectation of Success

Saari 1999	cide of interest. Genetic engineering has already been used for crop protection from (achiral) phenoxyacetate herbicides: cotton has shown enhanced tolerance towards 2,4-D when tfdA was transformed directly into the plant [15] or into root-associated microbes [16].
Saari 1998	contains would be protection of crops from mecoprop and dichlorprop. Cotton shows enhanced tolerance of 2,4-D in the presence of the pJP4-derived *tfdA* transformed either into the plant (Lyon et al. 1993) or into root-associated microbes (Feng and Kennedy 1997). For protection against the chiral phenoxypropionate herbicides, the pJP4-derived
'555 Patent	A wide variety of methods are available for introducing a gene encoding a desired protein into the target host under conditions that allow for stable maintenance and expression of the gene. These methods are well known to those skilled in the art and are described, for example, in U.S. Pat. No. 5,135,867.

EX1040, 93; EX1041, 422; 1036 Pet. 55-56; 1037 Pet. 55-56; 1038 Pet. 57; EX1001, 39:37-42; *see also* Ex. 1002, 39:19-24; EX1003, 39:32-37

CERTIFICATE OF SERVICE
(37 C.F.R. § 42.6(e))

The undersigned hereby certifies that on September 19, 2024, I caused a true and correct copy of the foregoing Petitioners' Demonstrative Exhibits to be served on the following via e-mail at:

Pier D. DeRoo	pier.deroo@ finnegan.com
Michael J. Flibbert	michael.flibbert@finnegan.com
Yieyie Yang	yieyie.yang@finnegan.com
Scott A. McKeown	SMcKeown-PTAB@WolfGreenfield.com
Jonathan B. Roses	JRoses-PTAB@WolfGreenfield.com

/Ben Picozzi/
Ben Picozzi
Reg. No. 76,862
Backup Counsel for Petitioners

Date: September 19, 2024