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Targeting *Magnaporthe oryzae* effector MoErs1 and host papain-like protease OsRD21 interaction to combat rice blast

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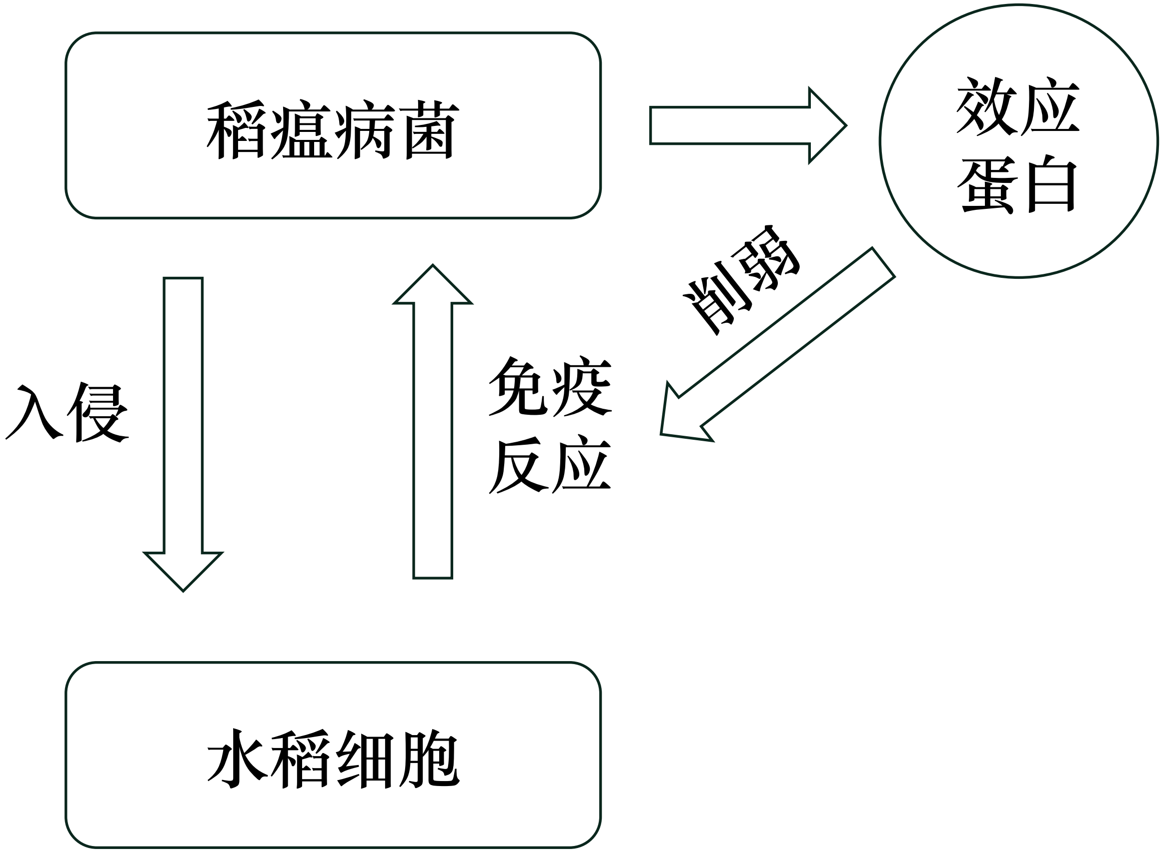
研究意义及相关生物学背景

■ 稻瘟病 (Rice Blast)

稻瘟病被称为“水稻癌症”，它是由稻瘟病菌 (Magnaporthe Oryzae)引起疾病，严重时可引起水稻减产40%～50%。

■ 稻瘟病菌致病的生物过程



抵抗稻瘟病的两种途径：

(1)从水稻稻瘟病抗性基因入手

(2)从稻瘟菌效应蛋白入手

(a) 效应蛋白-宿主细胞蛋白的相互作用机制

(b) 效应蛋白的序列、结构和功能

(c) 以效应蛋白为靶标，设计药物(复合物)

■ 以效应蛋白为靶标设计药物的局限性

(a) 效应蛋白质的保守性太差

(b) 稻瘟病菌的基因突变

(c) 药物失效

■ 目前的现状

多数杀菌剂是针对同一靶标开发，全球广泛使用的杀菌剂中60%的品种只针对3个靶标。

■ 本文的贡献

(1) We demonstrate that MoErs1, a species-specific effector protein secreted by the rice blast fungus Magnaporthe oryzae, inhibits the function of rice papain-like cysteine protease OsRD21 involved in rice immunity.

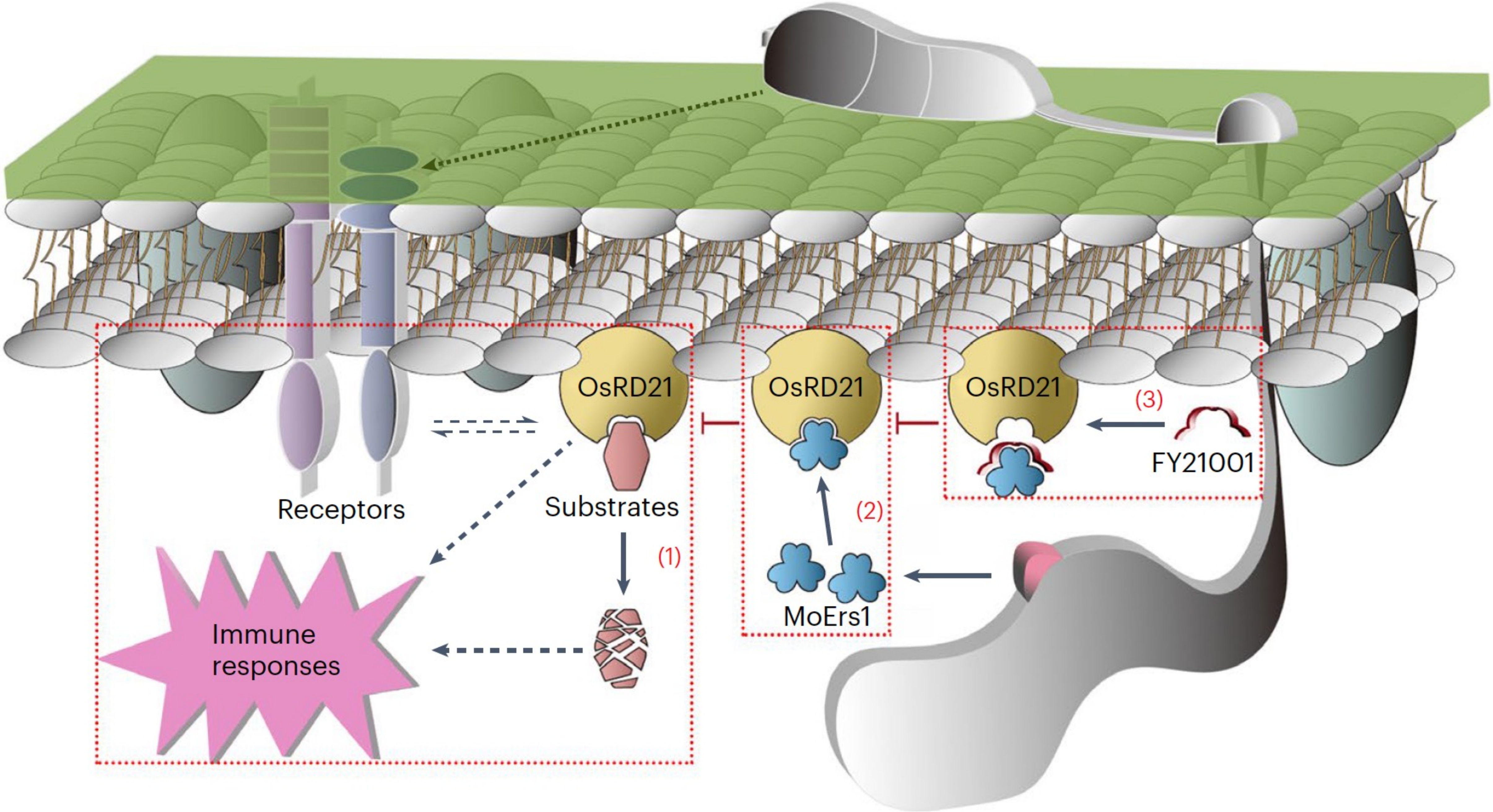
(2) Disrupting MoErs1-OsRD21 interaction effectively controls rice blast.

(3) We show that FY21001, a structure–function-based designer compound, specifically binds to and inhibits MoErs1 function.

(1) 证明了效应蛋白质MoErs1能够抑制蛋白质OsRD21的功能。

(2) 破坏MoErs1-OsRD21相互作用机制能够抑制稻瘟病。

(3) 设计了一种化合物FY21001，抑制MoErs1的功能。


OsRD21
OsRD21
OsRD21
(3)
FY21001
Receptors
Substrates
(2)
(1)
MoErs1
Immune
responses

实验结果及分析

- M. oryzae secretes effector MoErs1 to inhibit rice immunity (*)
- MoErs1 functions as a protease inhibitor that targets OsRD21

Biological Assembly 1

Explore in 3D: Structure | Sequence Annotations | Electron Density | Validation Report

Global Symmetry: Asymmetric - C1
Global Stoichiometry: Monomer - A1

Find Similar Assemblies

Biological assembly 1 assigned by authors.

Biological Assembly Evidence: gel filtration

Display Files | Download Files | Data API

7VS2

secreted fungal effector protein MoErs1

PDB DOI: https://doi.org/10.2210/pdb7VS2/pdb

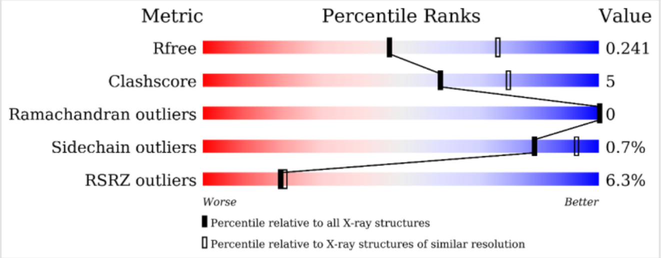
Classification: IMMUNE SYSTEM
Organism(s): Pyricularia oryzae
Expression System: Escherichia coli
Mutation(s): No

Deposited: 2021-10-25 **Released:** 2023-08-02
Deposition Author(s): Wang, F.F., Xing, W.M.
Funding Organization(s): Chinese Academy of Sciences

Experimental Data Snapshot

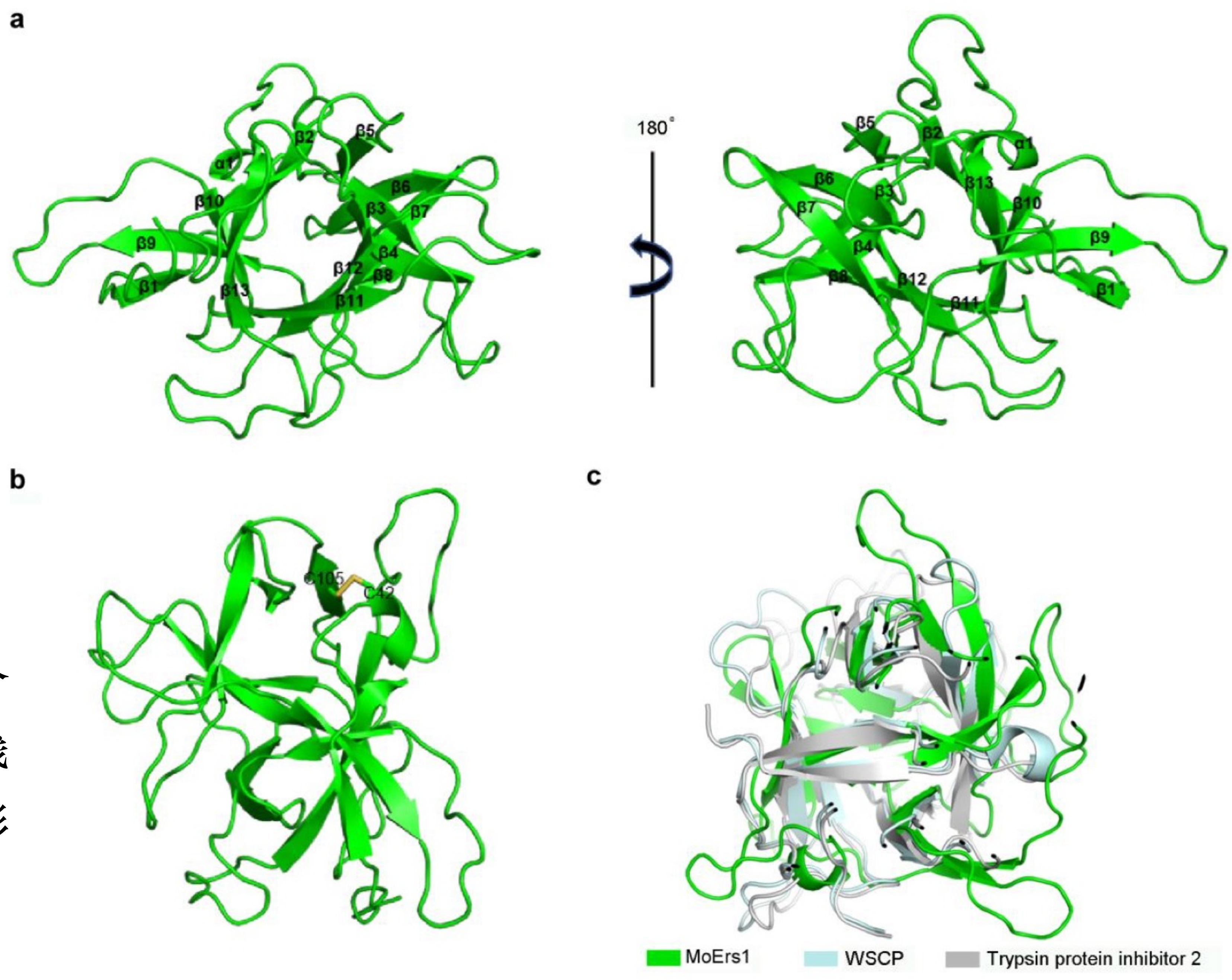
Method: X-RAY DIFFRACTION
Resolution: 2.50 Å
R-Value Free: 0.250
R-Value Work: 0.216
R-Value Observed: 0.217

wwPDB Validation 3D Report | Full Report



This is version 1.1 of the entry. See complete history.

>7VS2 | Chain A | MoErs1 | Magnaporthe oryzae (318829)
MPSTLEARALPQVSAVAK
PRACSSYPTFDPATGEATE
FIFYADSTEEPVAPFAGSV
VGKLANPNLAIARIGIAV
RGDLAKVVTKCFPDGGE
EGLRTRTHGDWRRLTLA
GGEDENIILIGQGPVAHRP
LTPHDHFFANGTQQPGVF
MGDNGSTTWAFSRKDAS
ASEPFDQYEIRLLKSADS
PLRNGEFRGFVRAA
(length: 194)



二硫键：两个半胱氨酸残基的巯基形成的共价键

(1) WSCP: water-soluble chlorophyll protein, 水溶性叶绿素蛋白

(2) Künitz-type protease inhibitor, 蛋白酶抑制剂

RMSD: 3.4 A

WSCP的功能：Inhibits the activity of Arabidopsis thaliana papain-like cysteine protease (PLCP) AtRD21.

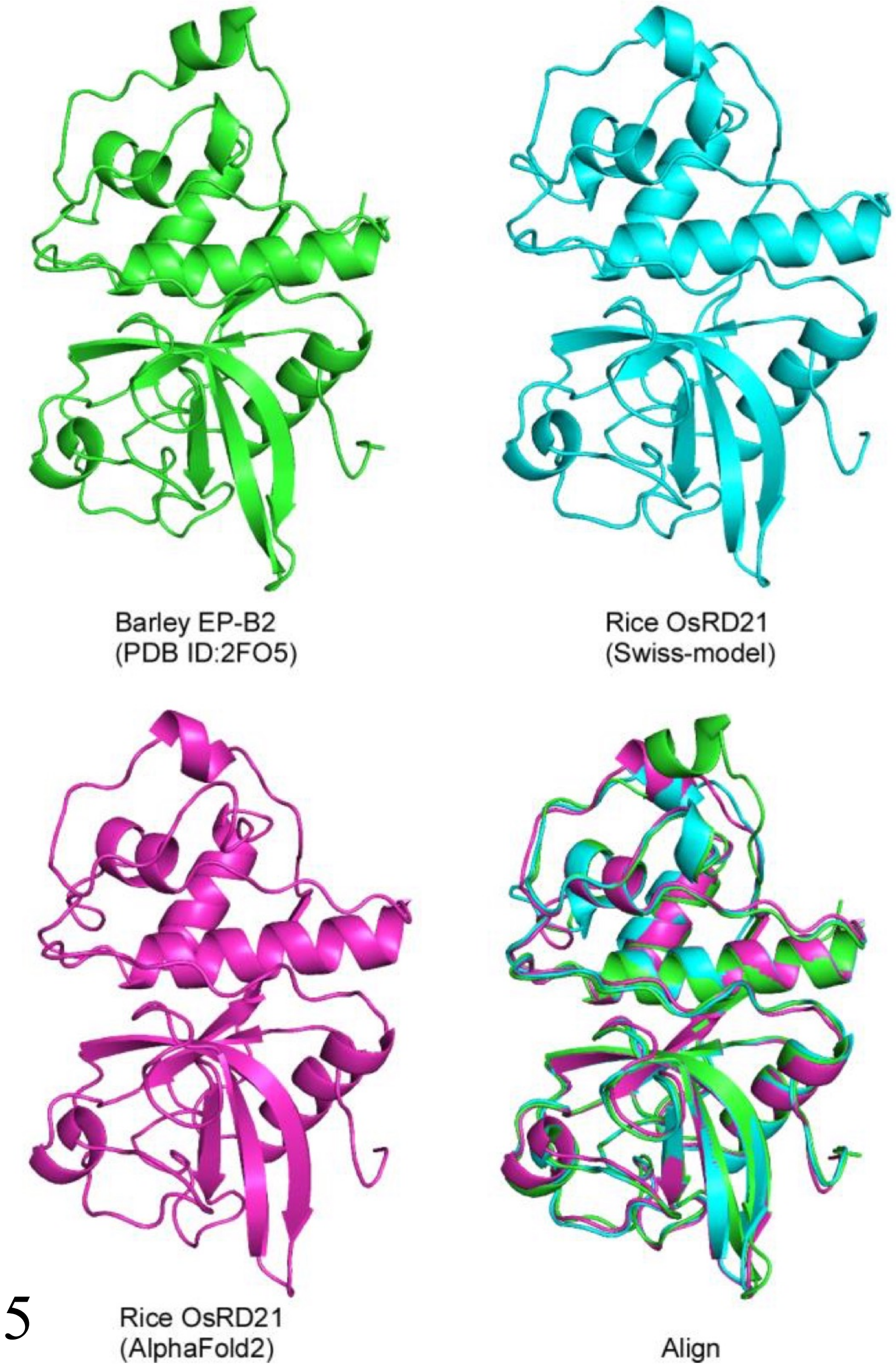
搜索 Oryza sativa L. Database，发现OsRD21 和AtRD21具有较高的同源性 (60.5%)。

生物实验证明:

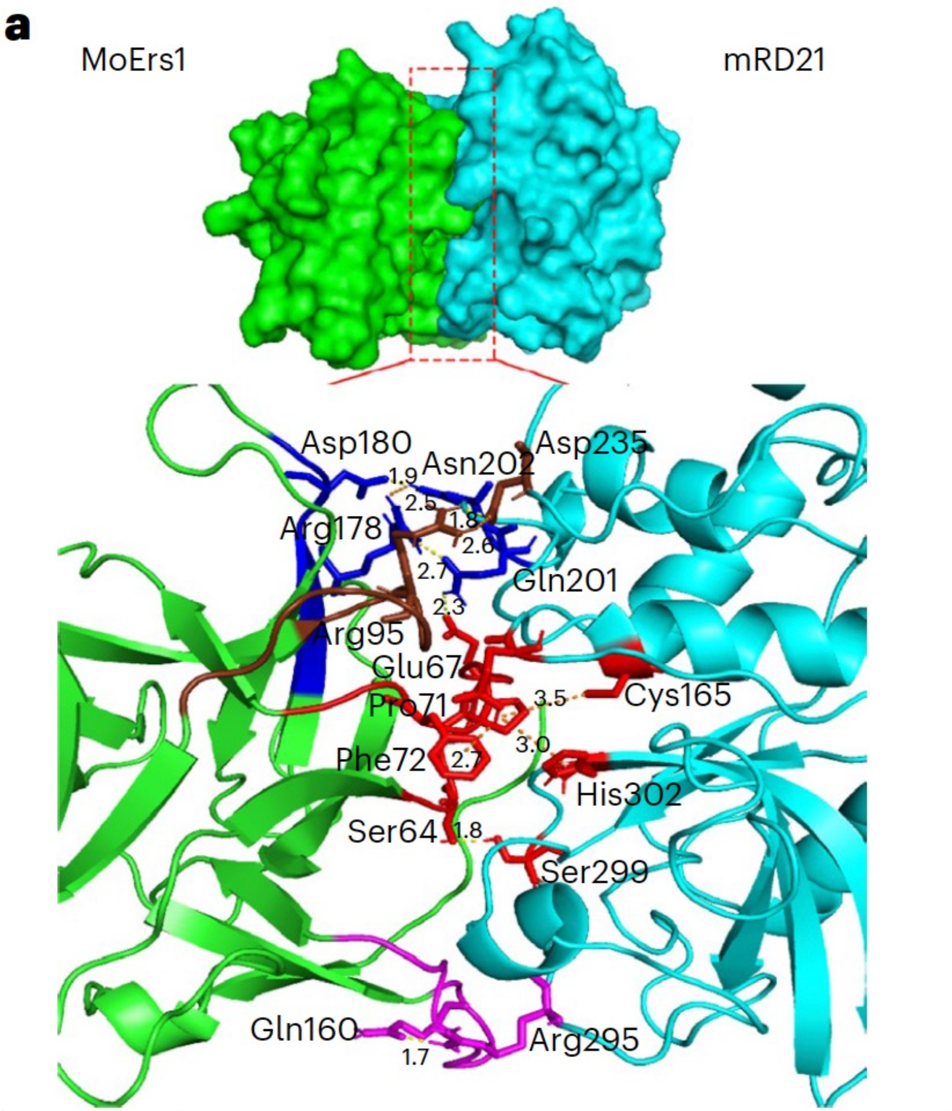
(1) OsRD21主要位于细胞膜上

(2) MoErs1 与 OsRD21能够相互作用，且相互作用发生在细胞膜上

■ MoErs1 inhibits the activity of OsRD21 to promote virulence



RMSD =0.545



Regions	Predicted interaction sites of MoErs1
L2	S64, E67, P71, F72 (red)
L4	R95 (chocolate)
L8	Q160 (magenta)
β11	R178, D180 (blue)

(1) 为了证明预测出的接触残基是准确的，作者将这些残基替换成其他类型的氨基酸，然后用生物实验验证，发现此时MoErs1–OsRD21 的相互作用不存在了。

(2) 生物实验证明： MoErs1通过绑定OsRD21 ，进而抑制了OsRD21的活性。

A structural model for the MoErs1–OsRD21 interaction predicted by ClusPro.

■ Diphenyl ether ester compounds (二苯醚酯化合物) inhibit MoErs1 function

验证MoErs1的保守性:

single-nucleotide polymorphism analysis

Supplementary Table 4. Sequence of *MoERS1* gene in various rice blast isolates.
>MGG_13009 70-15
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_13FM-16-1 885 13FM-16-1_contig_1602_361826:21748-22632
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_13FM-24-1 886 13FM-24-1_contig_419_195725:94350-95235
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_13FM-3-2 885 13FM-3-2_contig_782_91148:56881-55997
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_13FM-5-1 886 13FM-5-1_contig_1493_166360:101490-100605
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_13FM-9-1 885 13FM-9-1_contig_319_91170:56985-56101
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_2539_contig 886 2539_contig_13_212789bp_212789:16643-15758
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_81278_contig_v1_filtered 885 FJ81278_contig_10_1734154:294619-295502
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_81278_I_contig_filtered 885 81278_contig_1428_151212:56865-55982
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_98-06_contig 885 98-06_contig_378_112237bp_112237:69694-70578
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG
>MGG_13009_AV1-1-1 886 AV1-1-1_contig_94_312204:245737-246622
ATGCGCACCCAGTTCTCTCTCCTCGGAGTCGCGGCTCTCGCCAGCACCGTCGTCAACGCCATGCCCTCCACGCTCGAGGCCAGGGCCCTTCCCCAGGTTTCGGCCGTCGCCAAGCCGAG

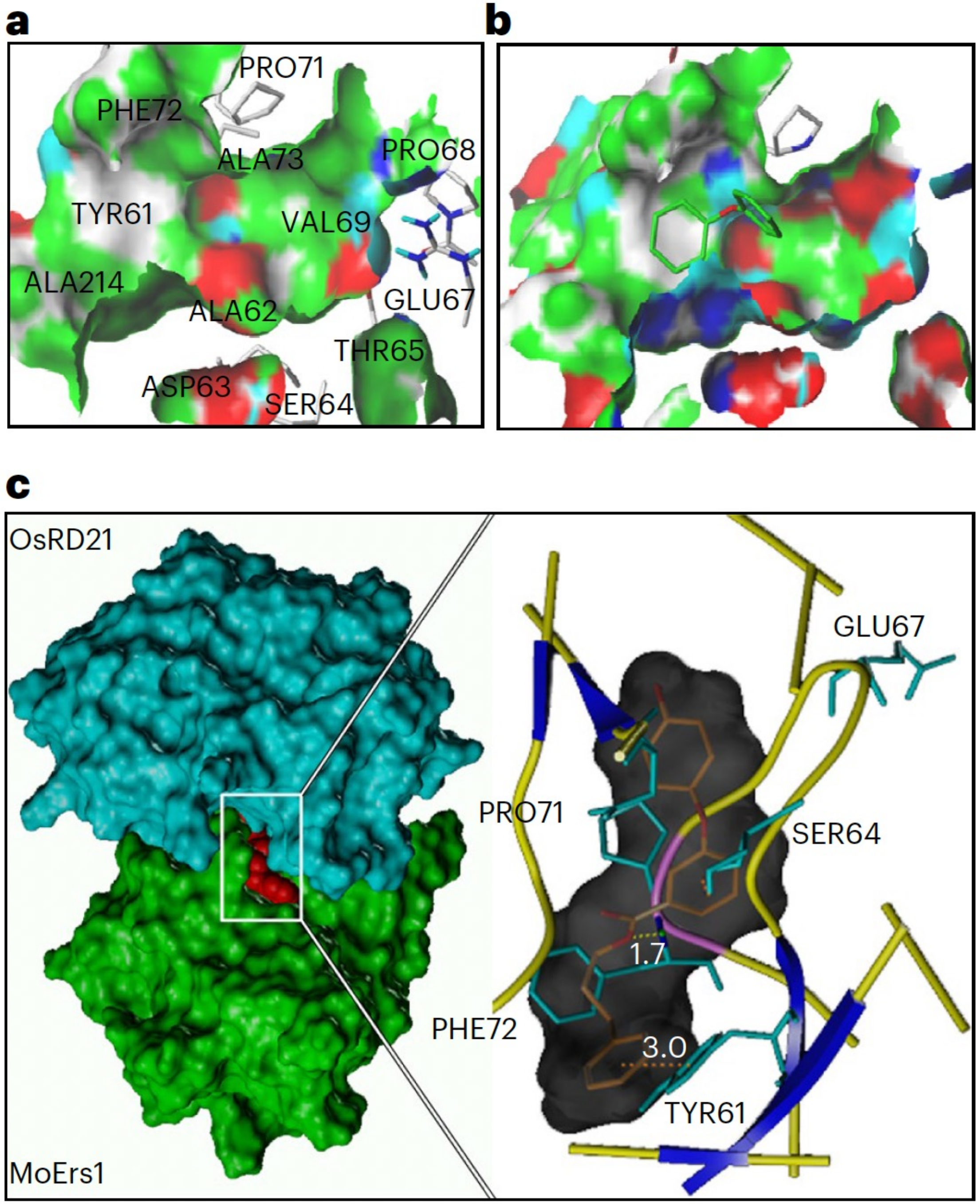
How to design small-molecule compounds to inhibit MoErs1 function ?

(1) 二芳基醚因其分子活性灵活在农药中被广泛使用。

(2) 二芳基醚骨架包含两个芳香环系统和一个灵活的氧桥。

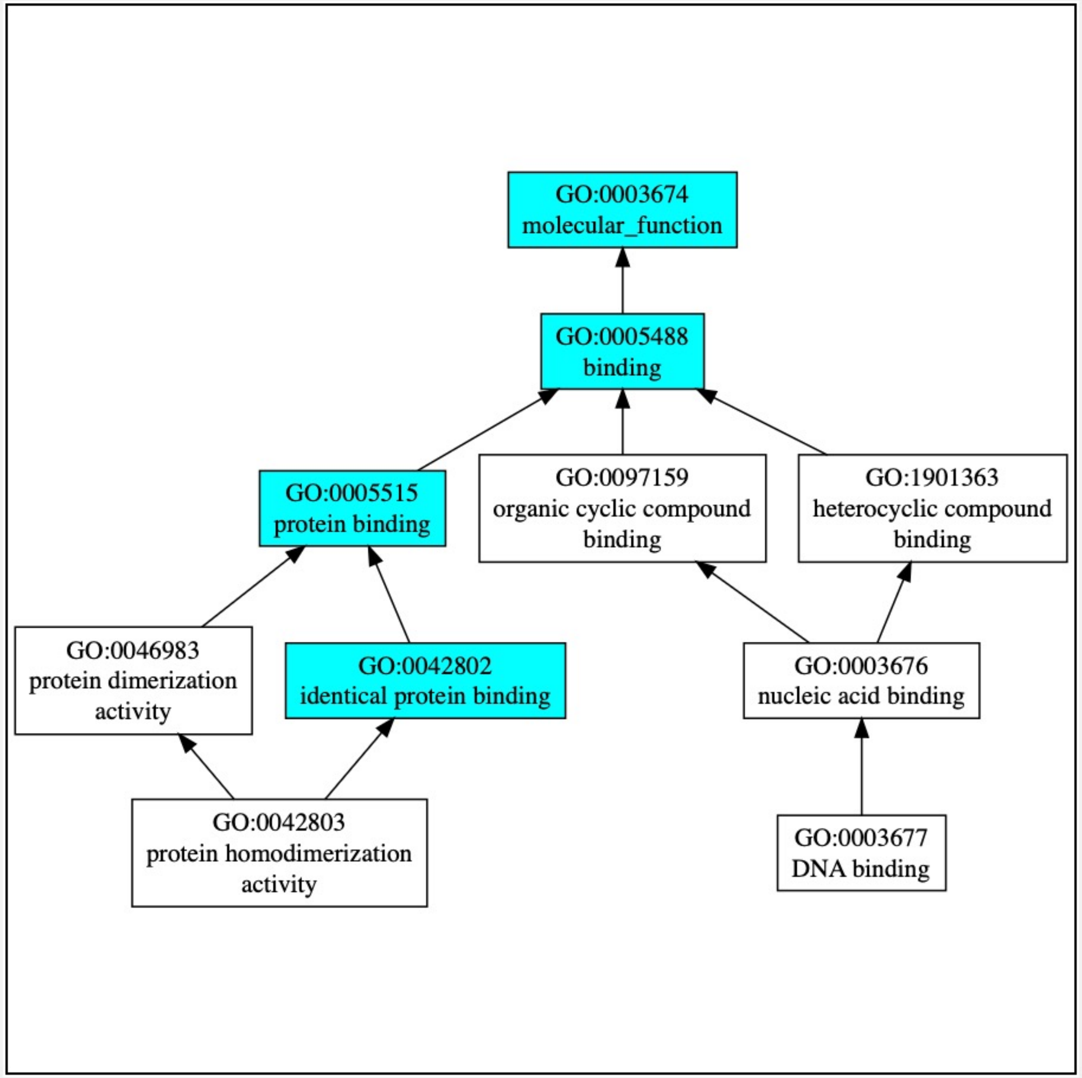
(3) 使用Sybyl-x-2.0分子对接分析二芳基醚–MoErs1时发现，氧原子与苯丙氨酸72的N-H形成氢键。此外，两个苯环分别朝向亲水区域（丝氨酸64，谷氨酸67）和疏水区域（苯丙氨酸72，酪氨酸61，脯氨酸71）。

(4) 为了增强抑制剂分子与靶标之间的结合稳定性，我们在二苯醚上引入了羟基和酯基。


a
b
c
PRO71
PHE72
ALA73
PRO68
TYR61
VAL69
ALA214
GLU67
ALA62
THR65
ASP63
SER64
OsRD21
MoErs1
GLU67
PRO71
SER64
1.7
PHE72
3.0
TYR61

我们可以做哪些工作

(1) 功能预测 (2) 蛋白质相互作用预测 (3) 分子对接 (4) 靶向药物设计



Molecular Function (MF)

GO term	$Cscore^{GO}$	Name
GO:0042802	0.528	identical protein binding
GO:0005515	0.528	protein binding
GO:0005488	0.528	binding
GO:0003674	0.528	molecular_function
GO:1901363	0.127	heterocyclic compound binding
GO:0097159	0.112	organic cyclic compound binding
GO:0046983	0.040	protein dimerization activity
GO:0003677	0.019	DNA binding
GO:0003676	0.019	nucleic acid binding
GO:0042803	0.013	protein homodimerization activity

Download full result of the above consensus prediction.

Click the graph to show a high resolution version.

(a) $Cscore^{GO}$ is the confidence score of predicted GO terms. Cs
better confidence in predicting the function using the templat

(b) The graph shows the predicted terms within the Gene Ont
color coded by $Cscore^{GO}$:

[0.40,0.5) [0.5,0.6) [0.6,0.7) [0.7,0.8) [0.8,0.9) [0.9,1.0]