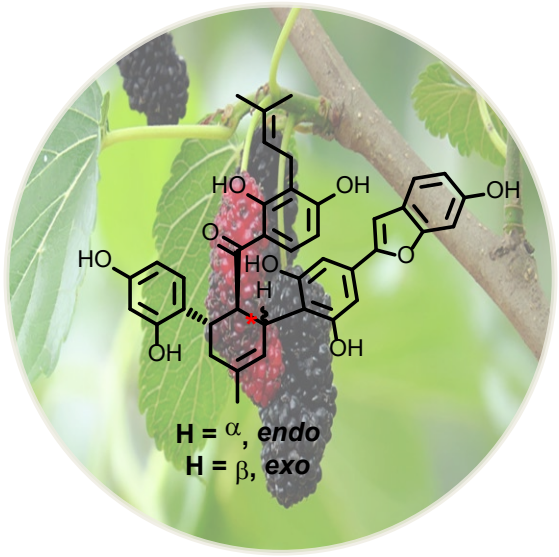
Discovery of First Standalone Intermolecular Diels-Alderase via Multi-omics Analysis and its Relationship between Structure and Function



Yang Jun

Jan. 23rd, 2021

G04A 杨军
导师： 雷晓光
研究方向： 高价值化学品的化学酶法合成以及新颖酶反应机制解析

G04C 甘亚琦
导师： 高宁
研究方向： 冷冻电镜技术和结构生物学

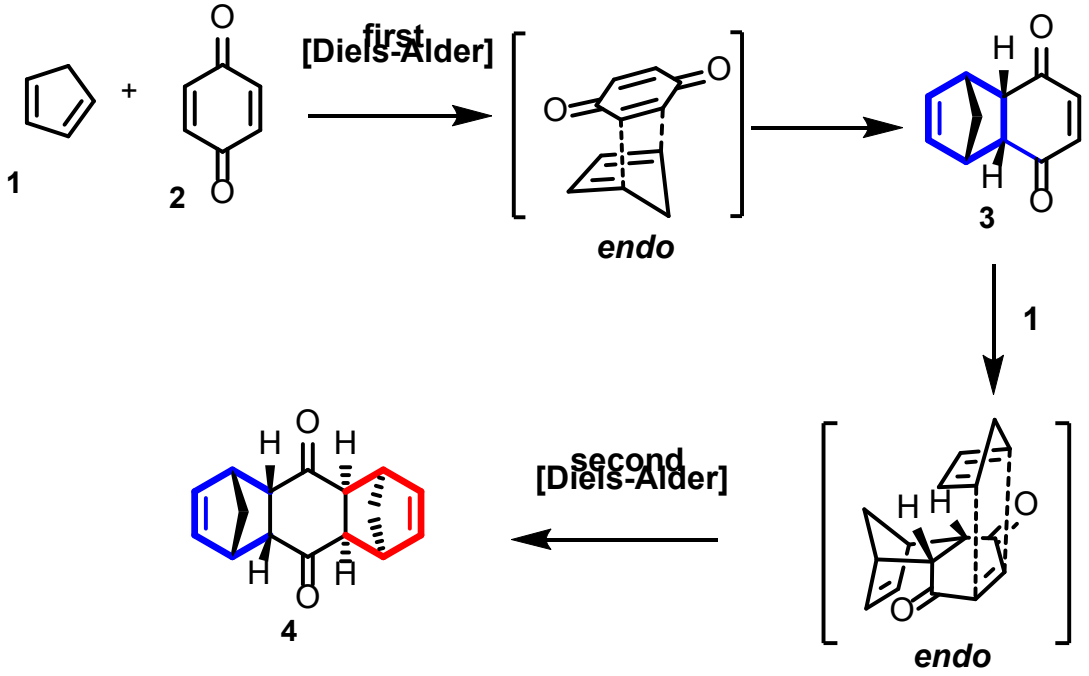
G04B 潘一格
导师： 秦跟基
研究方向： 植物学

G04D 康自红
导师： 刘君
研究方向： RNA表观遗传修饰在染色体结构改变和基因转录活性调控方面的机制

Diels-Alder reaction

Otto Diels (1876-1952)

Kurt Alder (1902-1958)

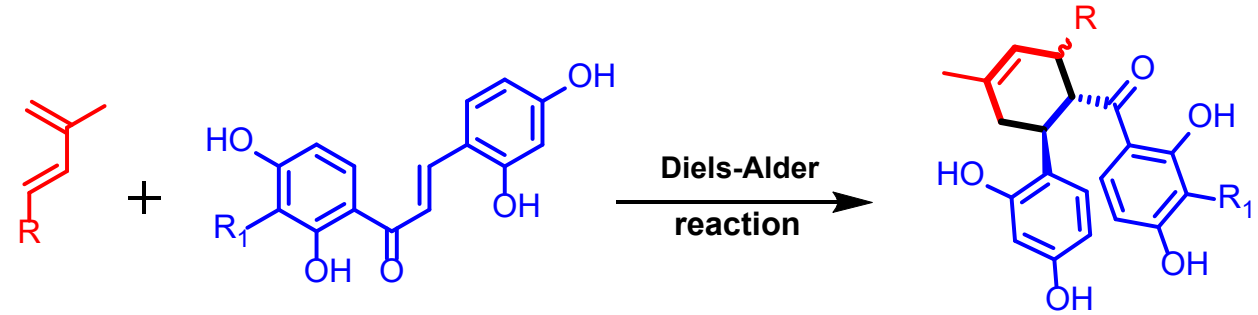


The landmark discovery of [4+2] cycloaddition reaction or Diels-Alder reaction in 1928 leads to the Nobel Prize in Chemistry in 1950.

"Thus it appears to us that the possibility of synthesis of complex compounds related to or identical with natural products such as **terpenes**, **sesquiterpenes**, perhaps even **alkaloids**, has been moved to the near prospect"

Diels, O.; Alder, K. *Justus Liebigs Ann. Chem.* **1928**, *460*, 98.

D-A type natural products from Moraceous plants

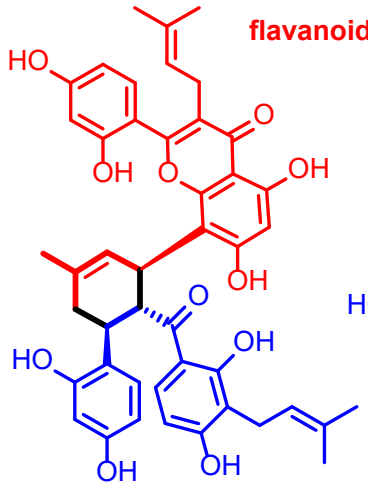


Dienes

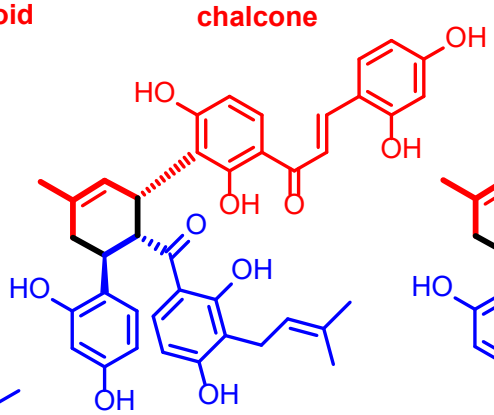
Dienophiles

> 80 D-A type products

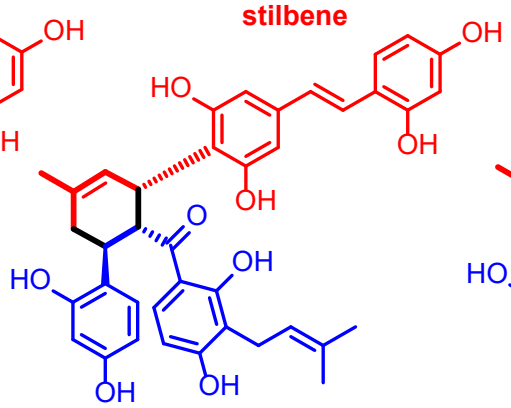
R = flavonoids, chalcones, stilbenes, 2-arylbenzofurans



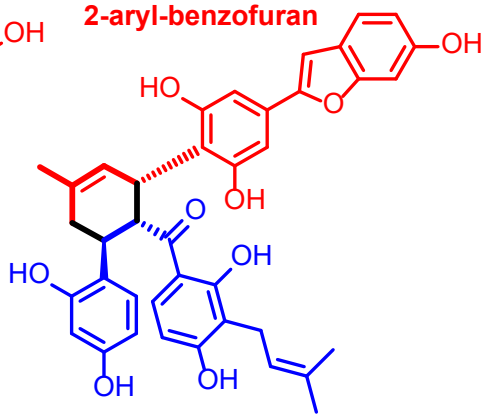
kuwanon H



kuwanon J



kuwanol E

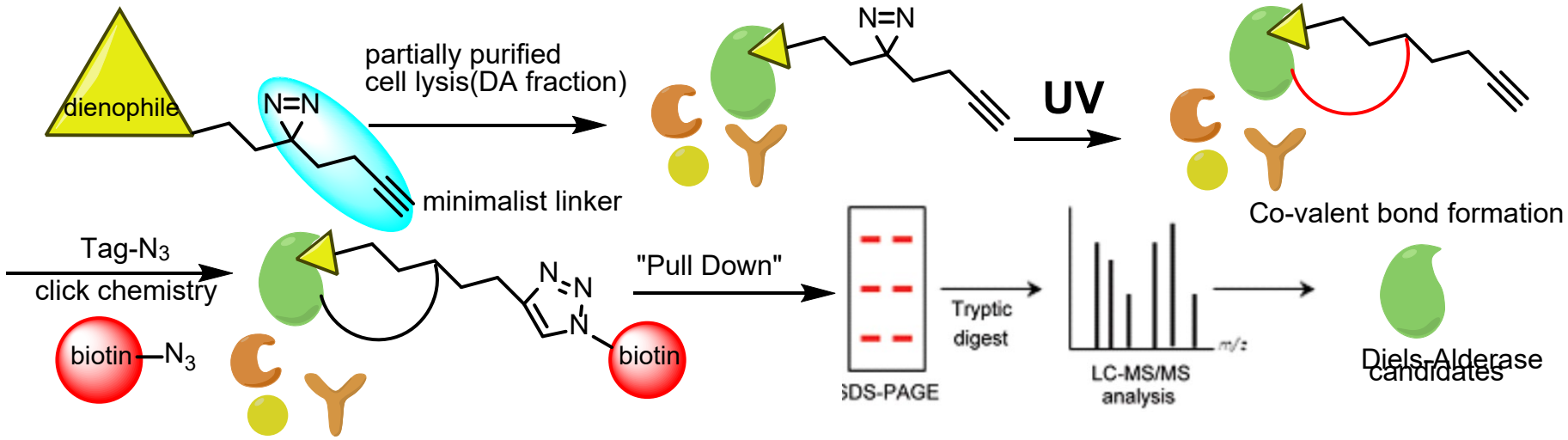


chalcomoracin

Nomura, T. *et al*. *Nat. Prod. Rep*. **1994**, *20*, 205.
Nomura, T. *et al*. *Pure Appl. Chem.* **1999**, *71*, 1115.

Our new strategy for identification of DAase in Morus alba

- Target identification using biosynthetic intermediate probes（BIPs）

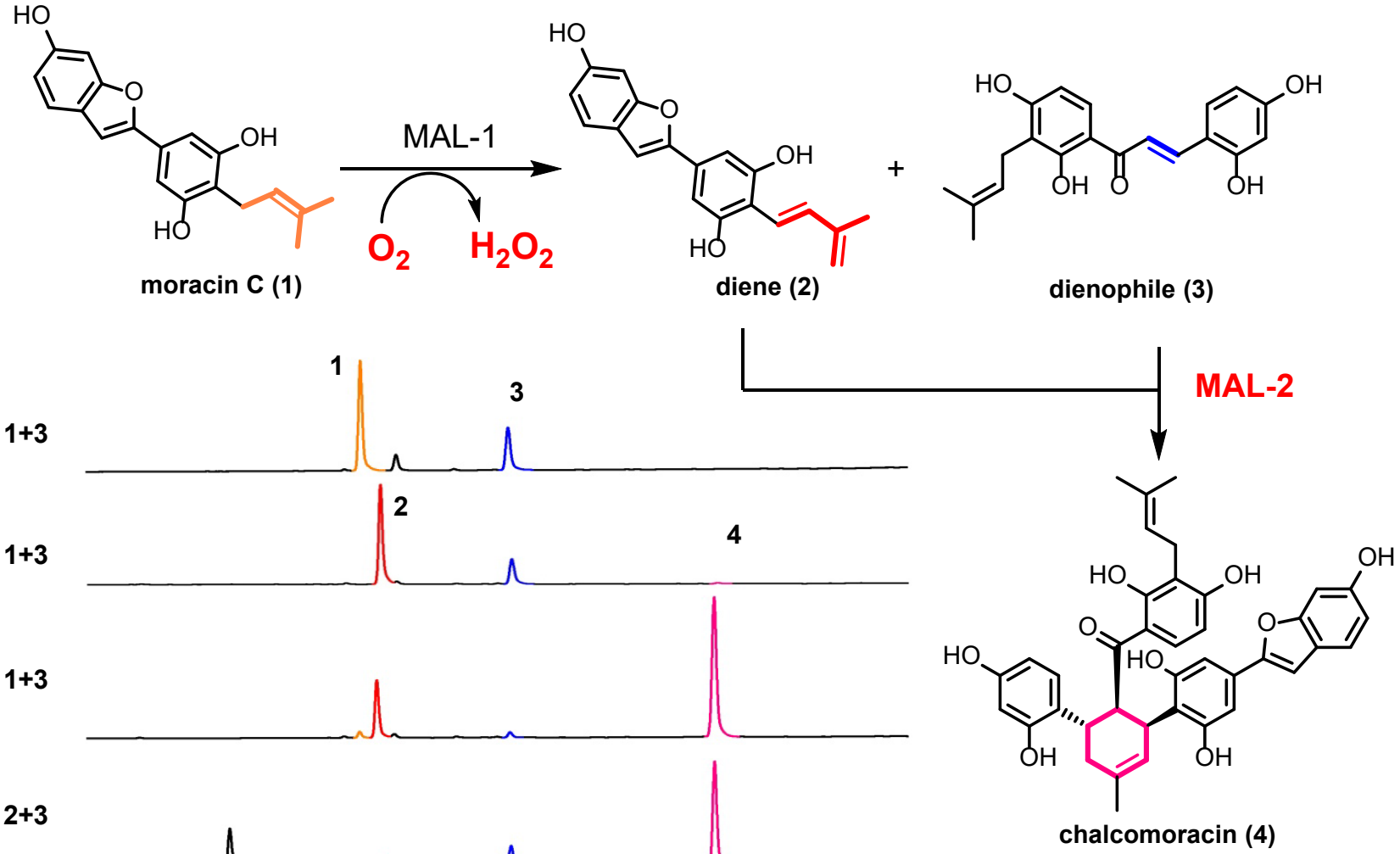


Yao, S. Q. *et al. Angew. Chem. Int. Ed.* **2013**, *52*, 8551.

- Combination of three different methods

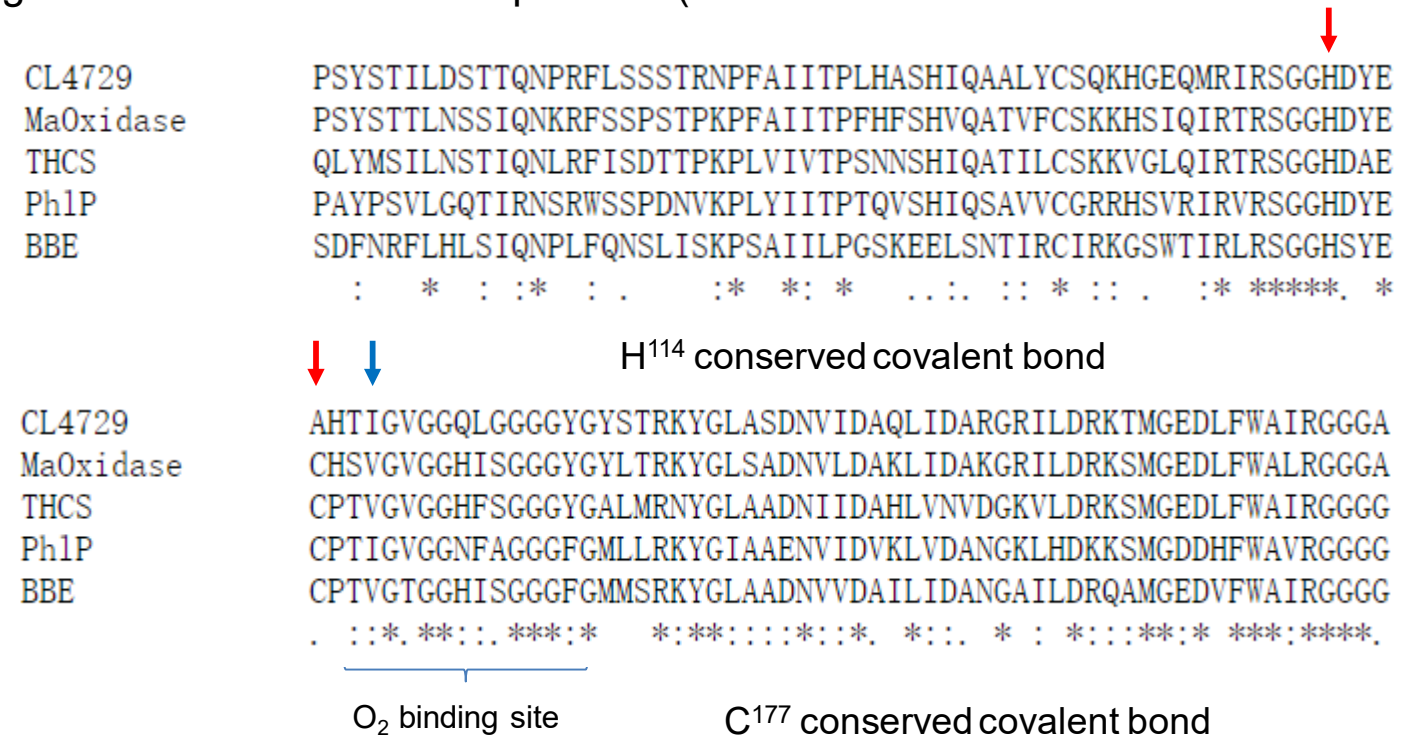
Biochemistry approach (Enzyme purification)	Chemical Biology approach (Target identification using biosynthetic intermediate probes)	Genetics approach (transcriptome analysis)
➢ Improve abundance ➢ Decrease off-targets	➢ Target ID	➢ Gene sequence information ➢ Transcriptional level

MAL-2 is a functional Diels-Alderase



Sequence alignment reveals the difference between the oxidase and DAase

Multi-alignment of known BBE like proteins (unusual bi-covalent attachment of FAD)

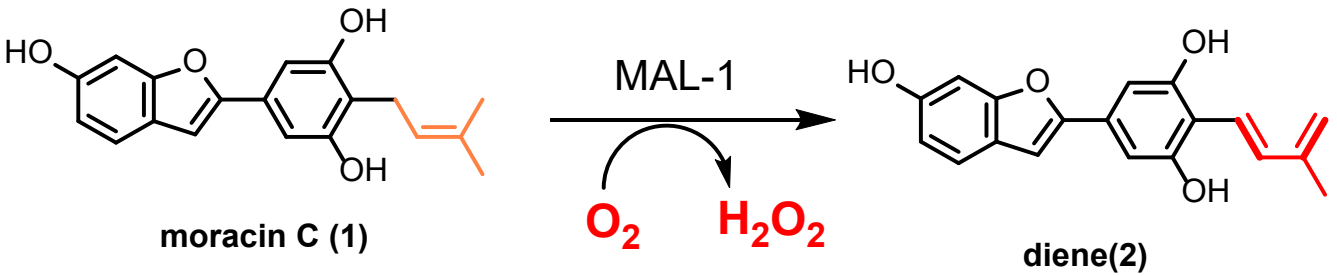


- C is covalently linked to FAD, mutation of C to A may decrease the redox potential of FAD.

- V is the gatekeeper of O_2 binding site, mutation of V to I will lose the oxidase's activity: Phl P4 is dehydrogenase which uses quinone as electron acceptor. Mutation of I to V increase the oxidase's activity by 60,000 fold.

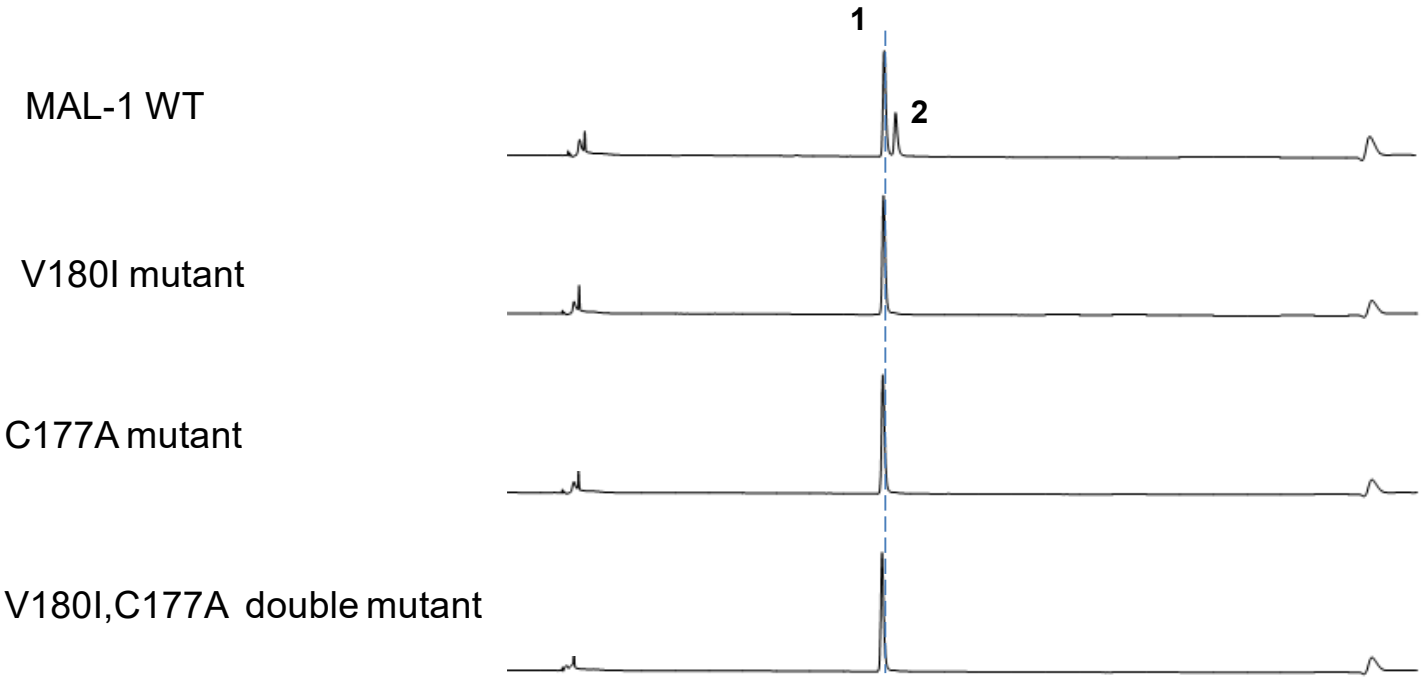
Zafred, D. et al. *FEBS Journal*, **2015**, *282*, 3060.

Mutation of the oxidase at the O_2 binding site



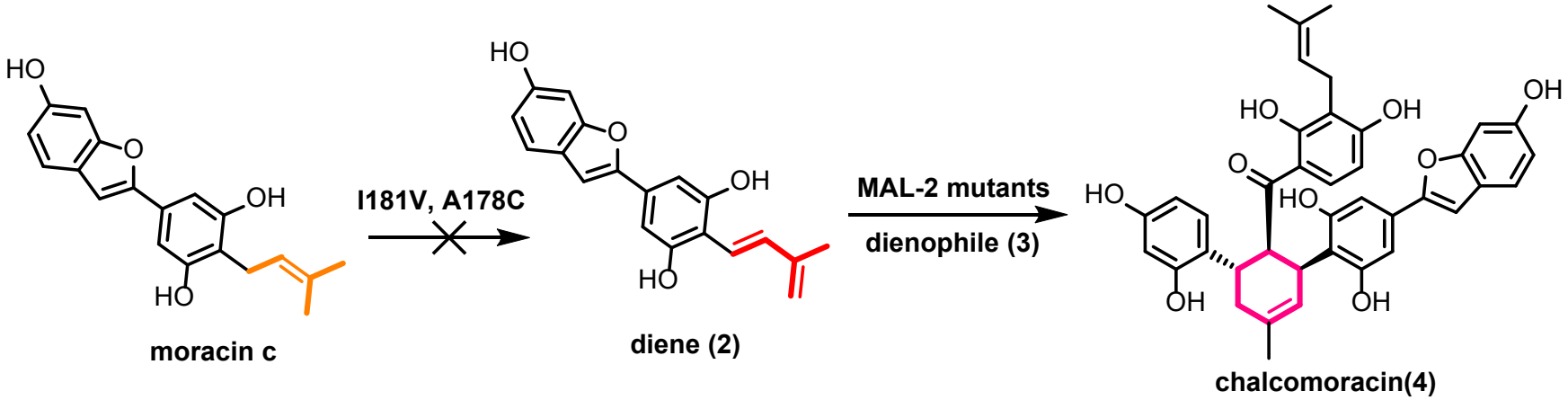
moracin C (1)

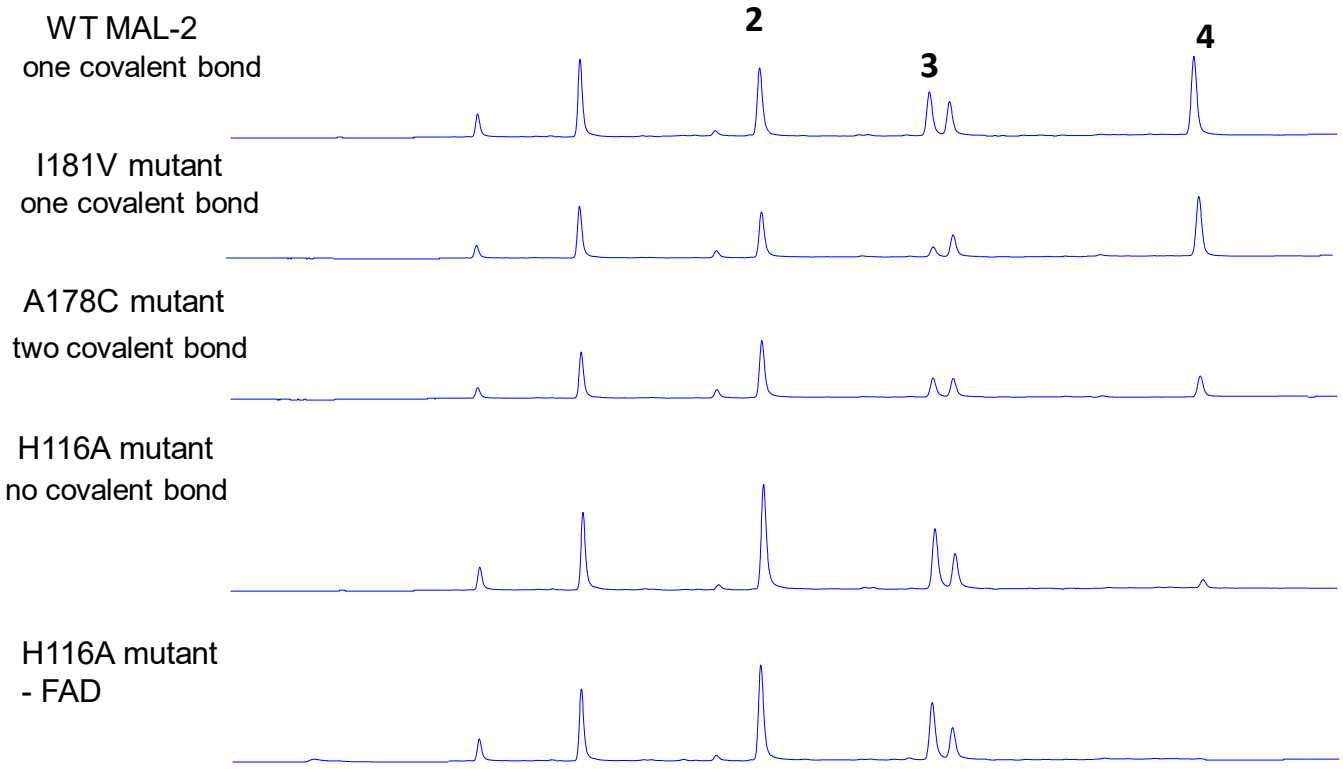
diene(2)



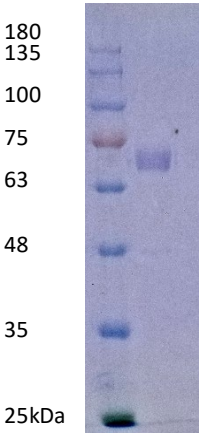
- Mutation of C^{177} or V^{180} decreases the oxidation activity, no diene was observed.
- All these three mutants don't have activity of DAase.

Mutation of the DAase(MAL-2)

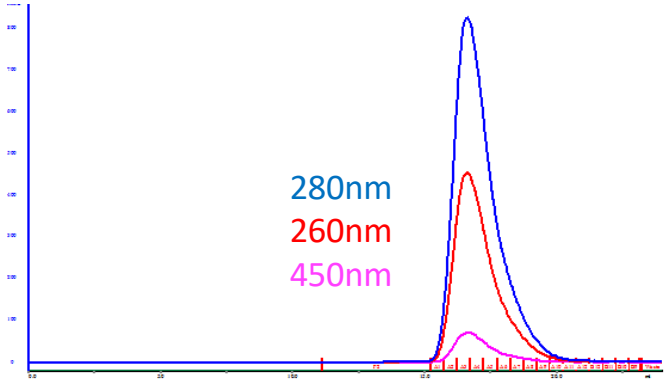




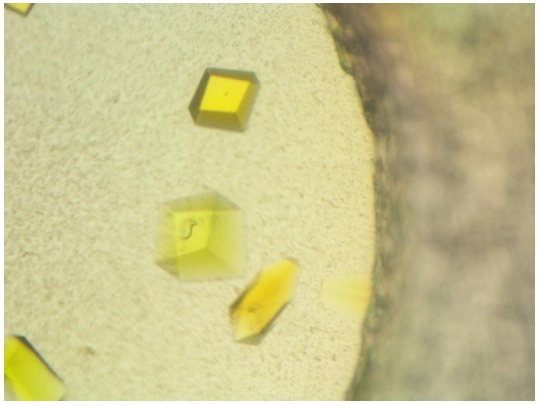
Apo-structure of MAL-2



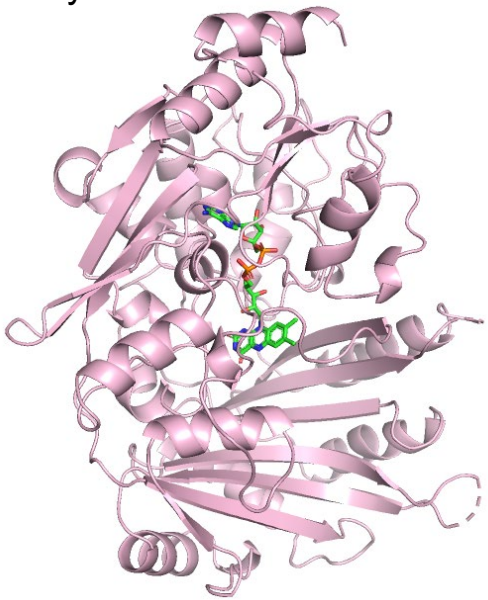
Expression in insect cell
baculovirus expression system



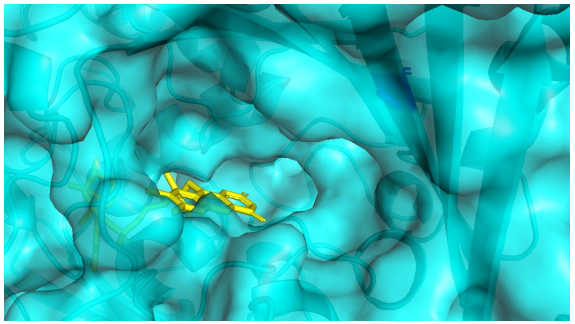
Gel filtration by Superdex 200 column



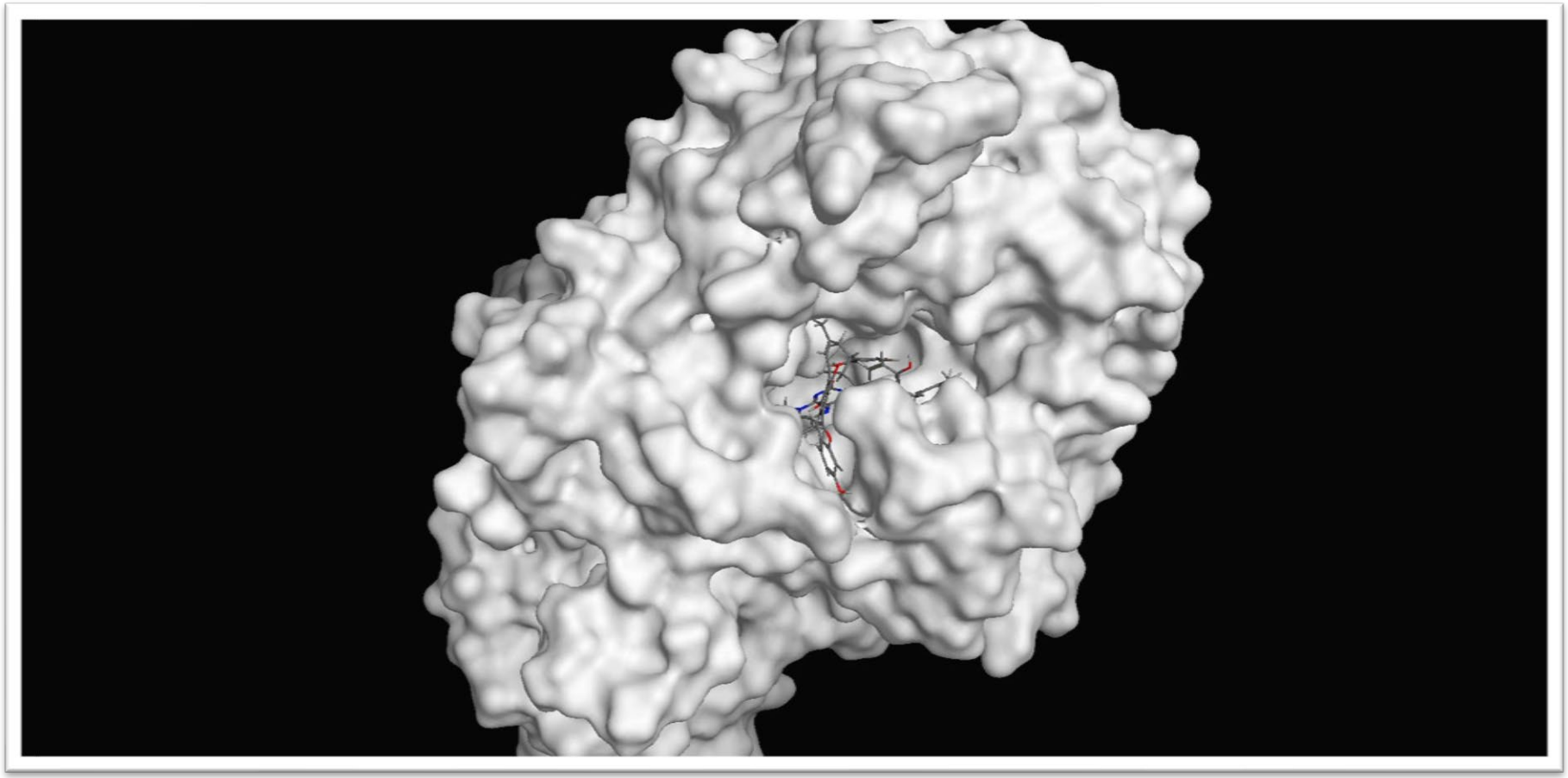
Crystal screen



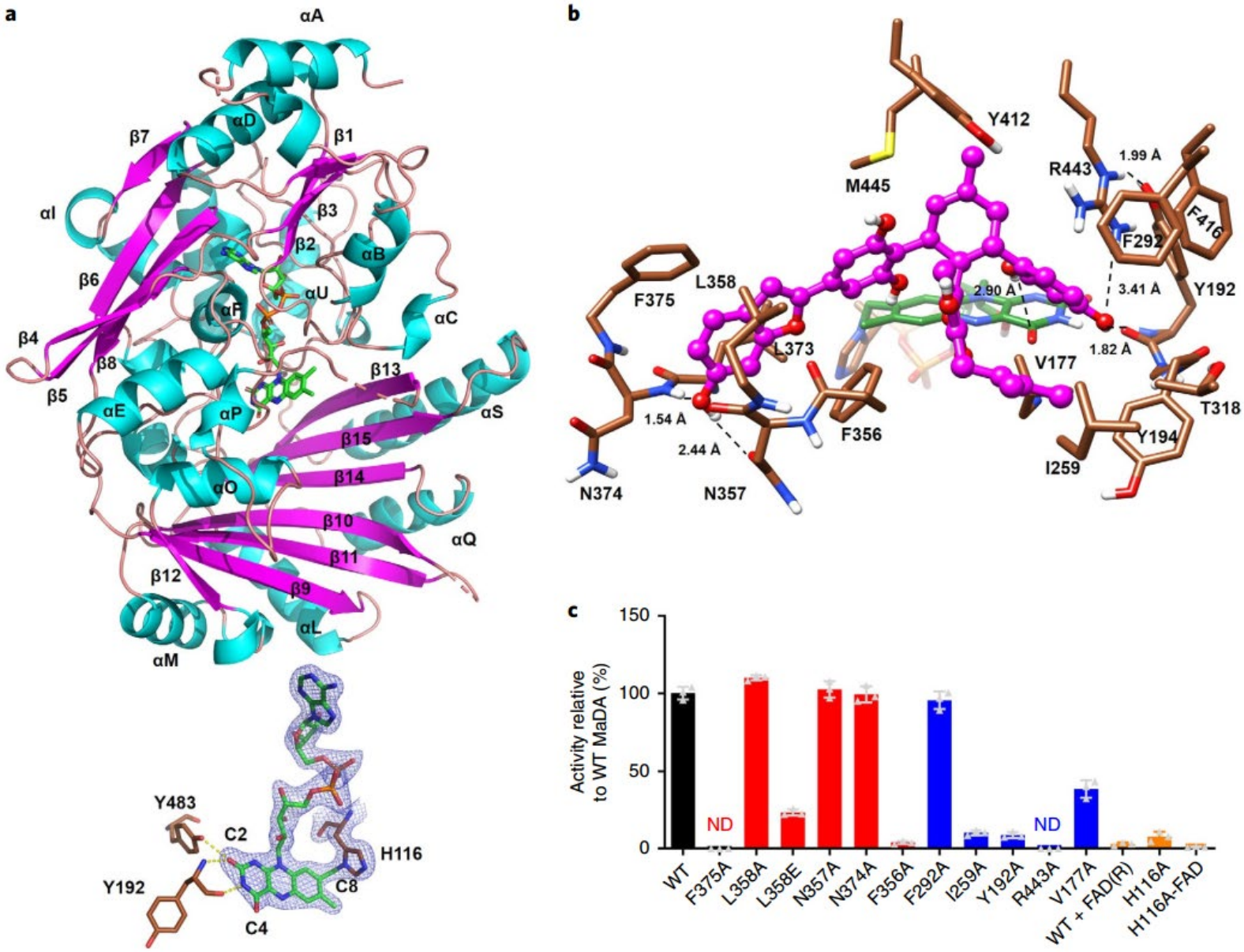
Monomer 2.3 Å



Complex structure of MAL-2_chalcomoracin



Relationship between Structure and Function



Gao, L.; Lei, X.G. *Nat. Chem.* **2020**, *12*, 620.

Summary

- Identity several oxidases in the *Morus alba* cell callus
- Identity the first standalone intermolecular Diels-Alderase
- Elucidate the structure of MAL-2 and its's possible mechanism
- Elucidate the relationship between structure and function of MAL-2

Acknowledgement

Thanks for Listening