Based on LmrR Scaffold: Artificial Metalloenzyme (ArM) Catalyzed Asymmetric Organic Reactions

Reporter : Jie Lin
Advisor: Prof. Shengming Ma
Date: 20200515

1. Background

2. ArM Catalyzed Asymmetric Organic reactions
- The Copper Enzyme Catalyzed reactions
- The Iron Enzyme Catalyzed reactions
- The Bimetallic Enzyme Catalyzed reactions

3. Summary and outlook

1. Background

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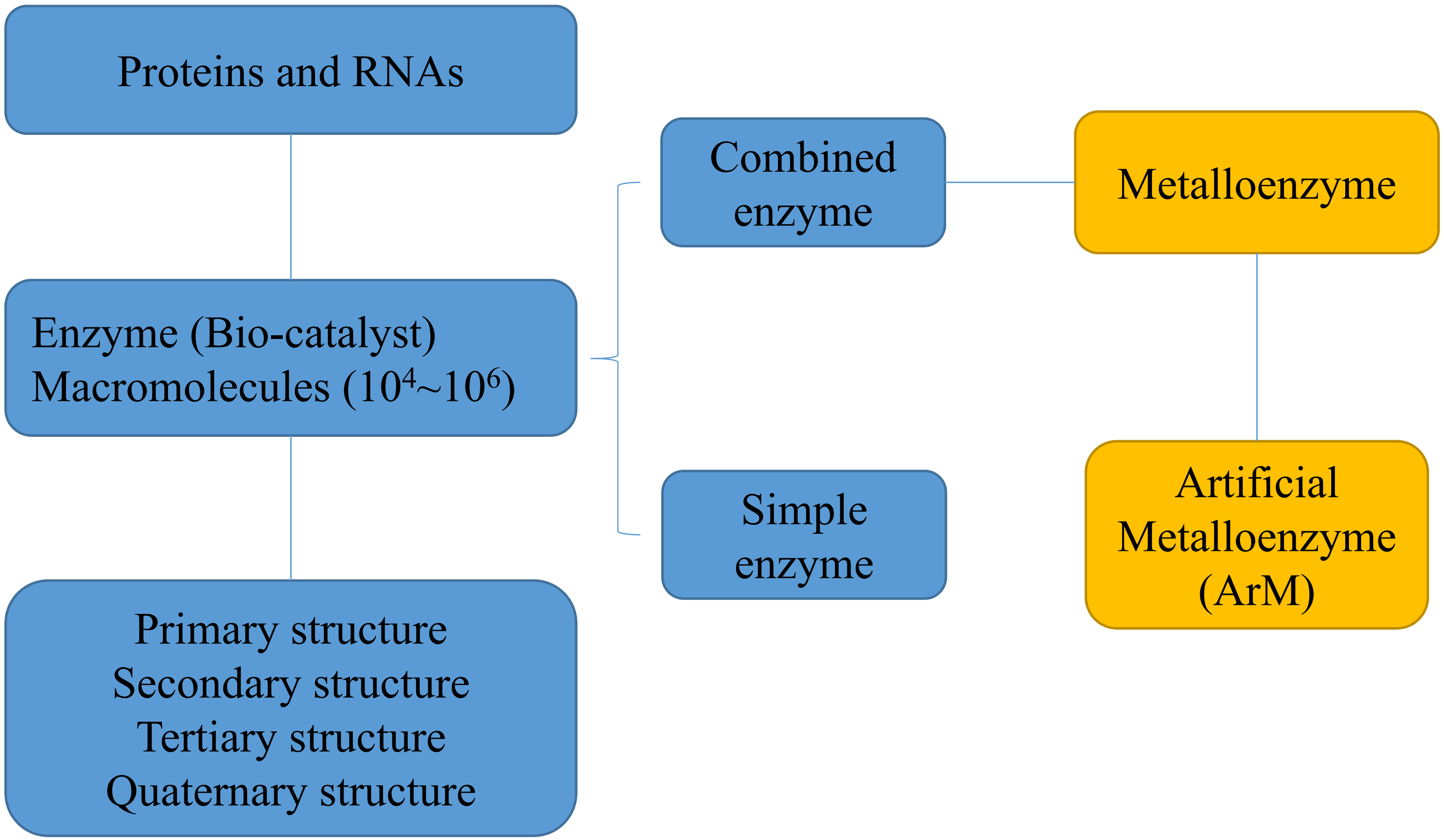
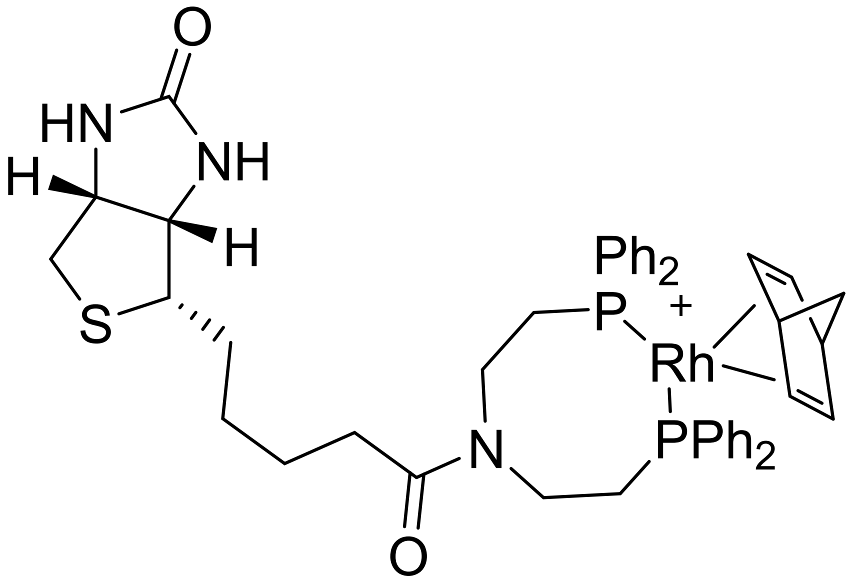
Enzymes

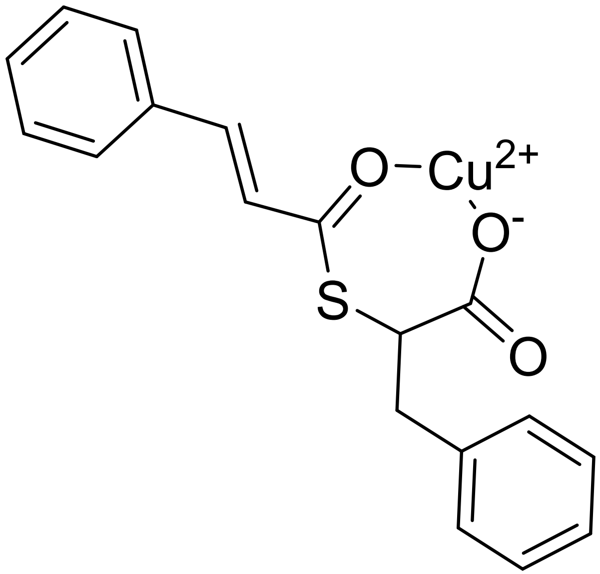


Proteins and RNAs
Enzyme (Bio-catalyst)
Macromolecules (10^4~10^6)
Primary structure
Secondary structure
Tertiary structure
Quaternary structure
Combined enzyme
Simple enzyme
Metalloenzyme
Artificial Metalloenzyme (ArM)

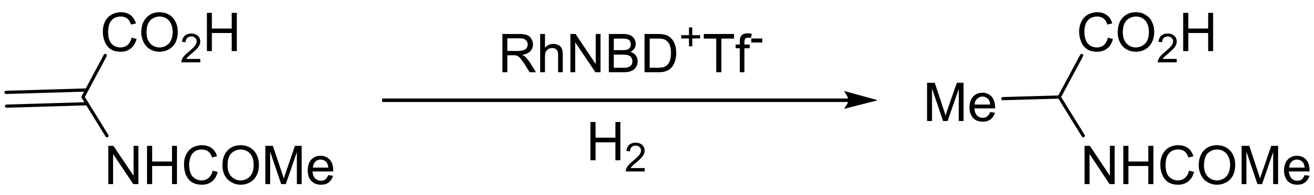
The earliest ArM



RhNBD^{+}Tf^{-}

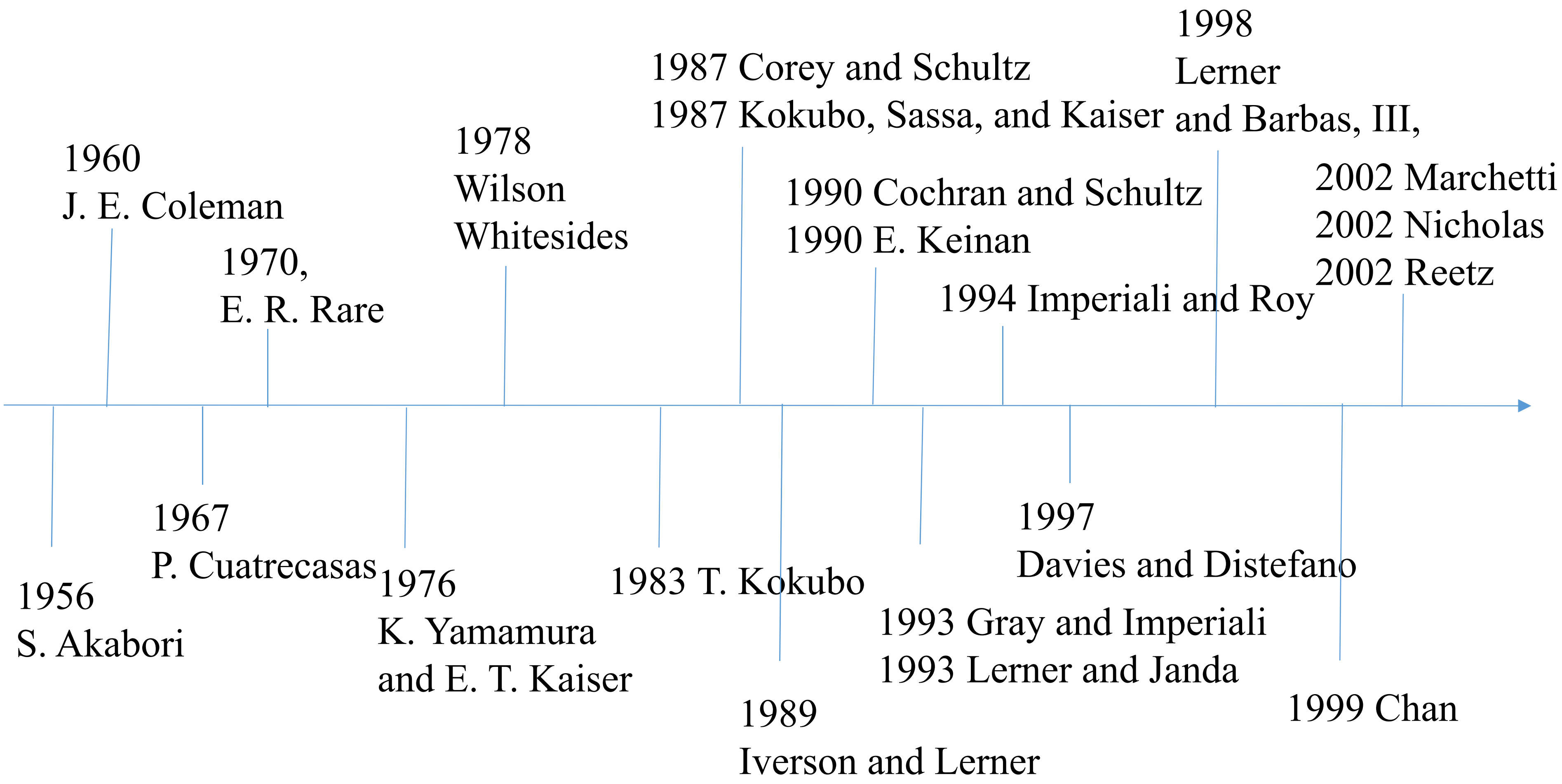


Cu Carboxypeptidase A

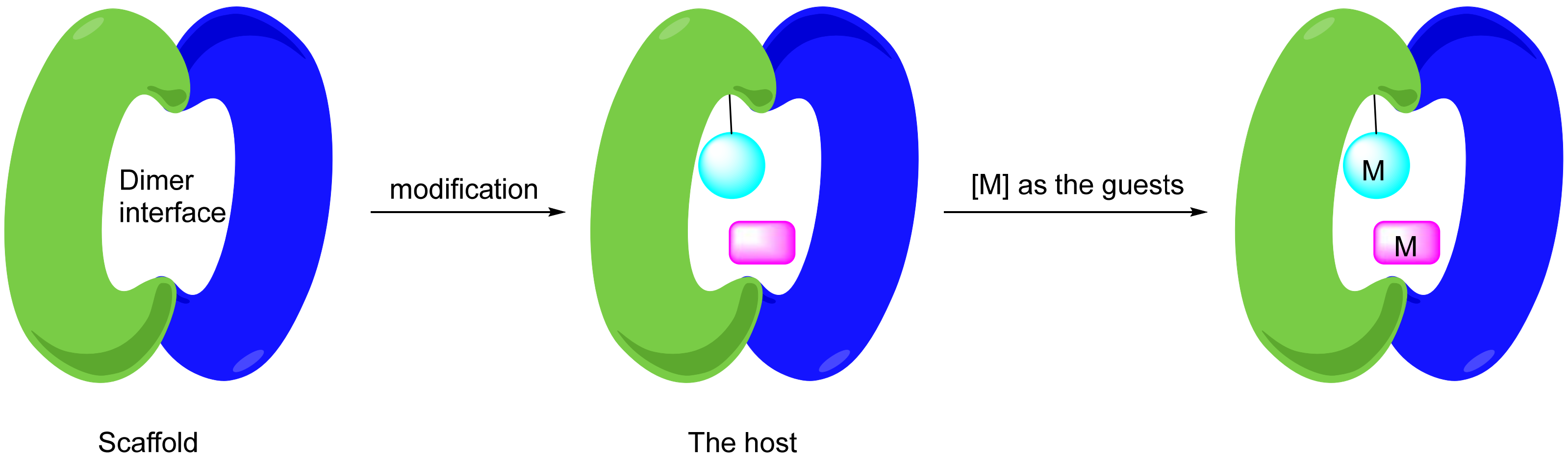


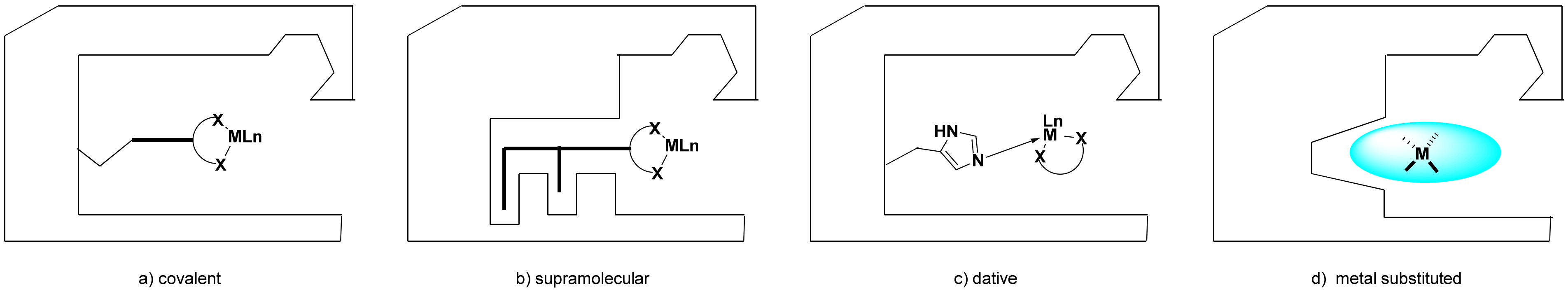
Yamamura, K.; Kaise, E. T. *J. Chem. Soc. Chem. Commun.* **1976**, 830.
Wilson, M. E.; Whitesides, G. M. *J. Am. Chem. Soc.* **1978**, *100*, 306.

The ArM of Historical Contributions



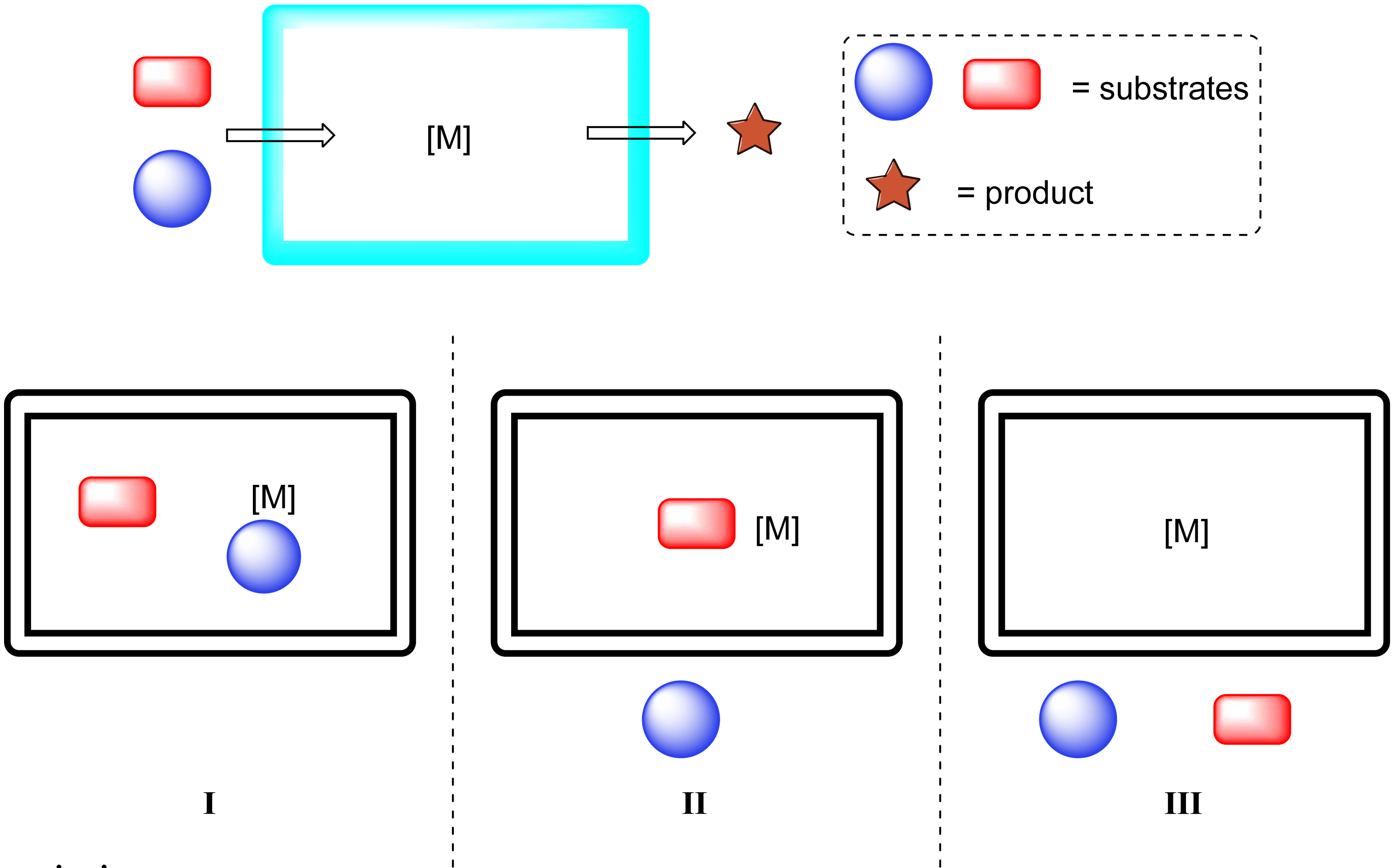
The structure of ArM





Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Köhler, J. C.; Ward, T. R. *Chem. Rev.* **2018**, *118*, 142
Roelfes, G. *Acc. Chem. Res.* **2019**, *52*, 545

Reaction mode

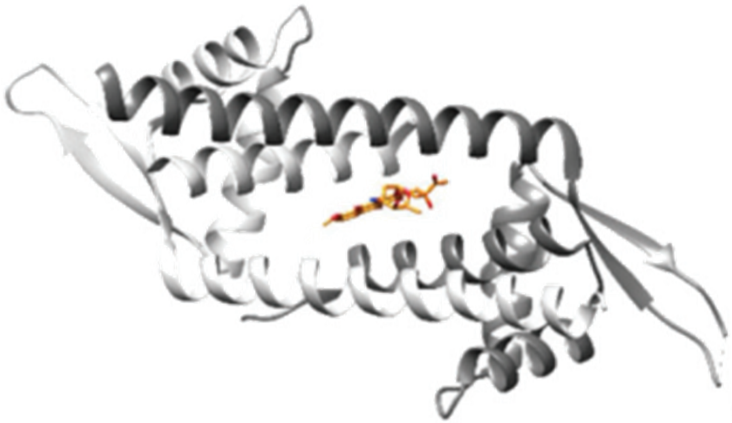


Characteristic:

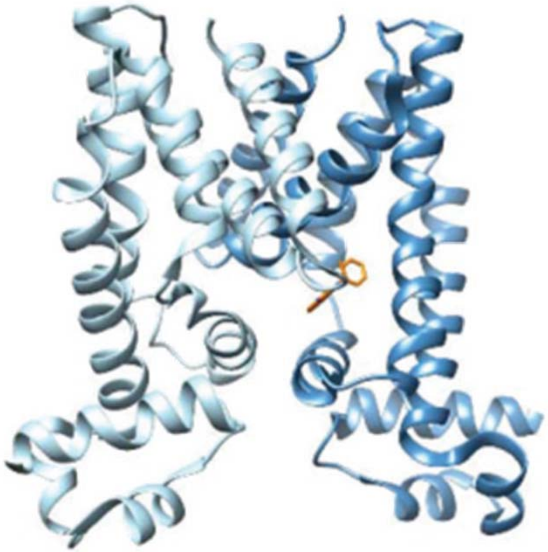
- Secondary coordination sphere could interact with metal catalysts, substrates, or intermediates
- Generated from protein scaffolds with inherent functionality
- Endow homogeneous catalysts with a genetic memory

Schwizer, F.; Okamoto, Y.; Heinisch, T.; Gu, Y.; Pellizzoni, M. M.; Lebrun, V.; Reuter, R.; Köhler, V.; Köhler, J. C.; Ward, T. R. *Chem. Rev.* **2018**, *118*, 142

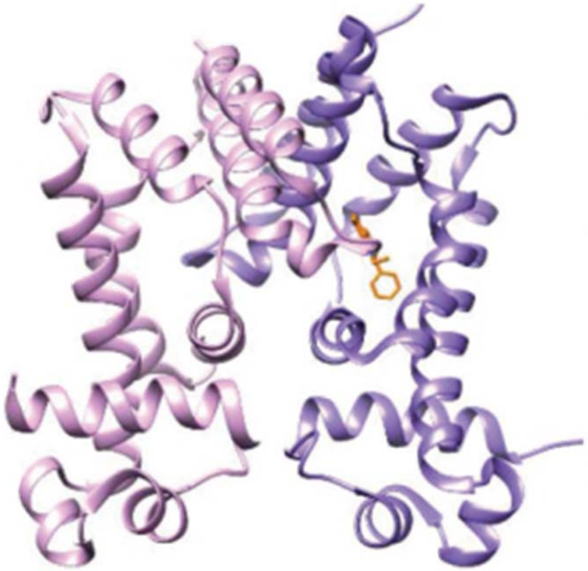
The scaffold of ArM



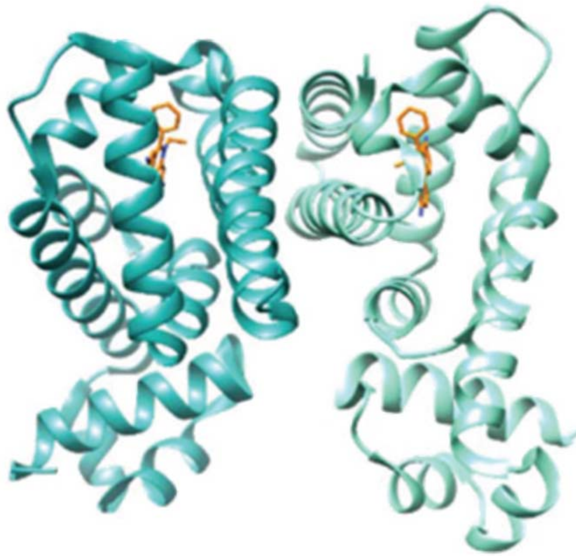
LmrR



QacR

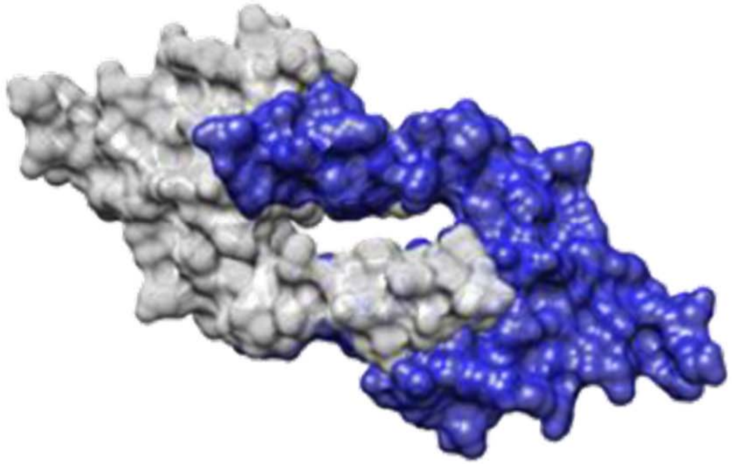


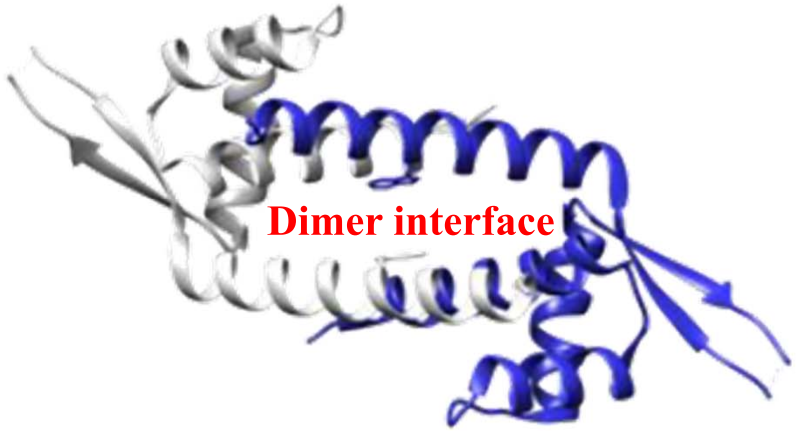
CgmR



RamR

Lactococcal Multidrug Resistance Regulator (LmrR)





Bersellini, M.; Roelfes, G. *Org. Biomol. Chem.* **2017**, *15*, 3069.

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2. ArM Catalyzed Asymmetric Organic reactions
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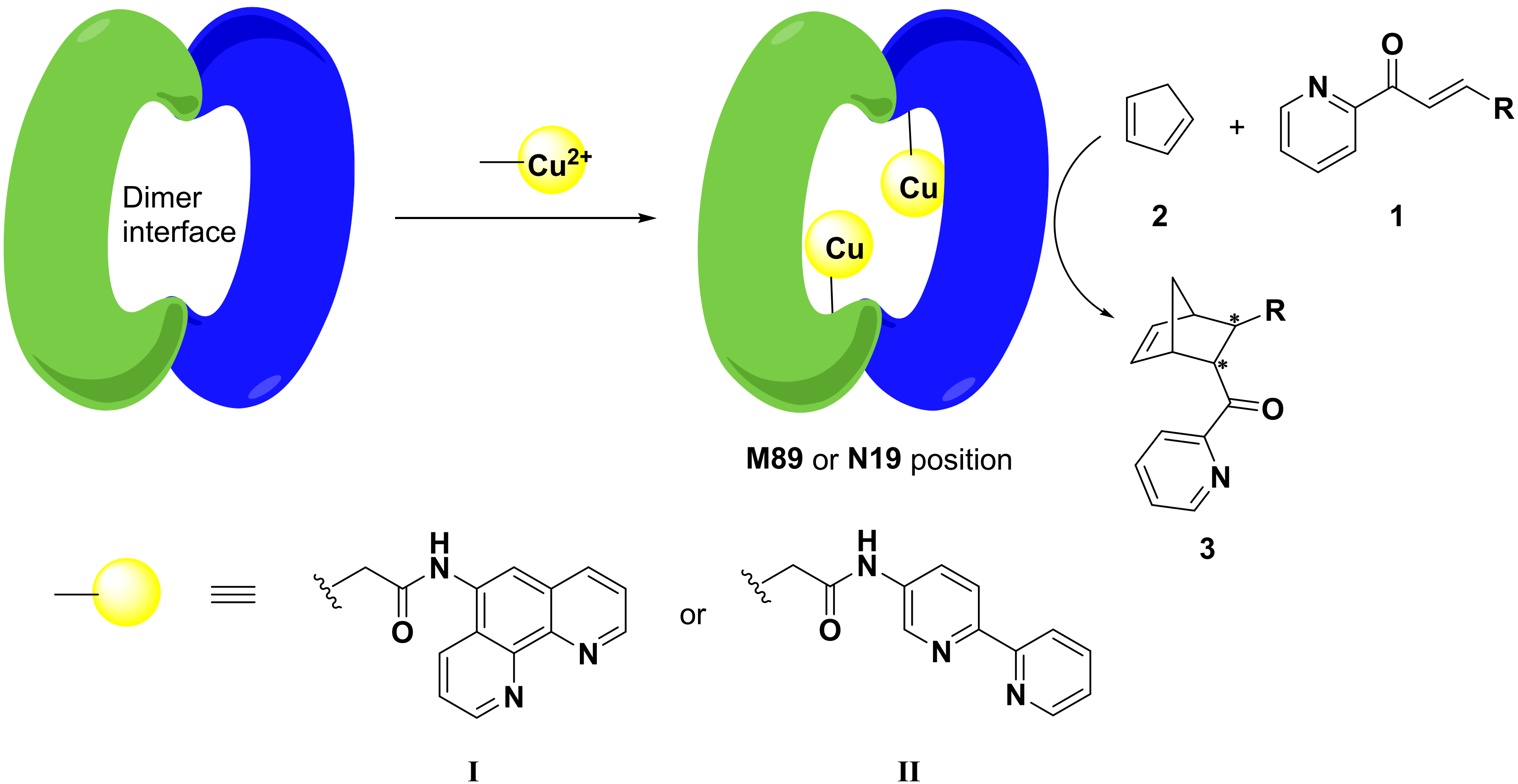
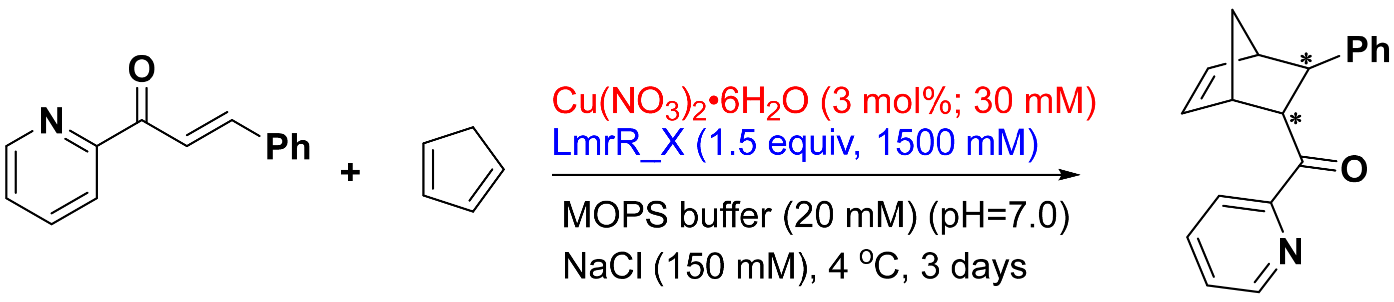
The Copper Enzyme Catalyzed D-A reactions



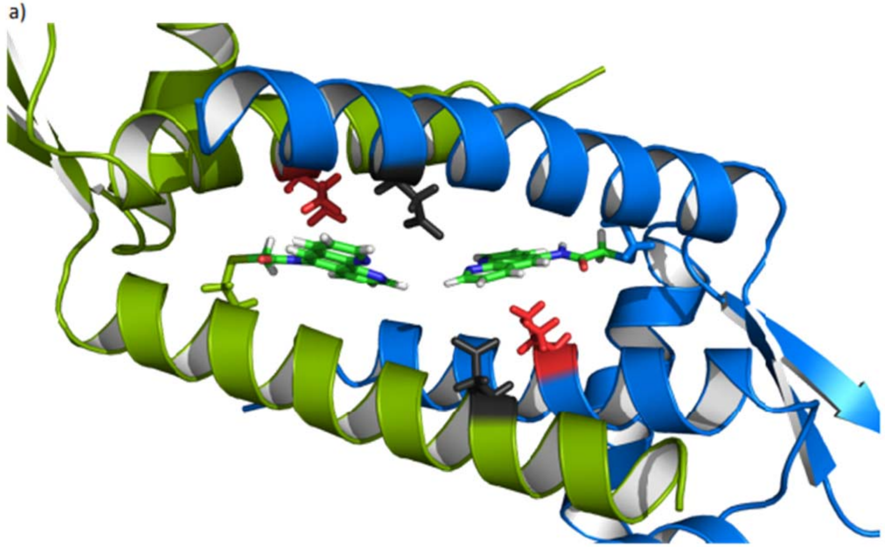
Bos, J.; Fusetti, F.; Driessen, A. J. M.; Roelfes, G. *Angew. Chem. Int. Ed.* **2012**, *51*, 7472.

Optimizations and Scope Study

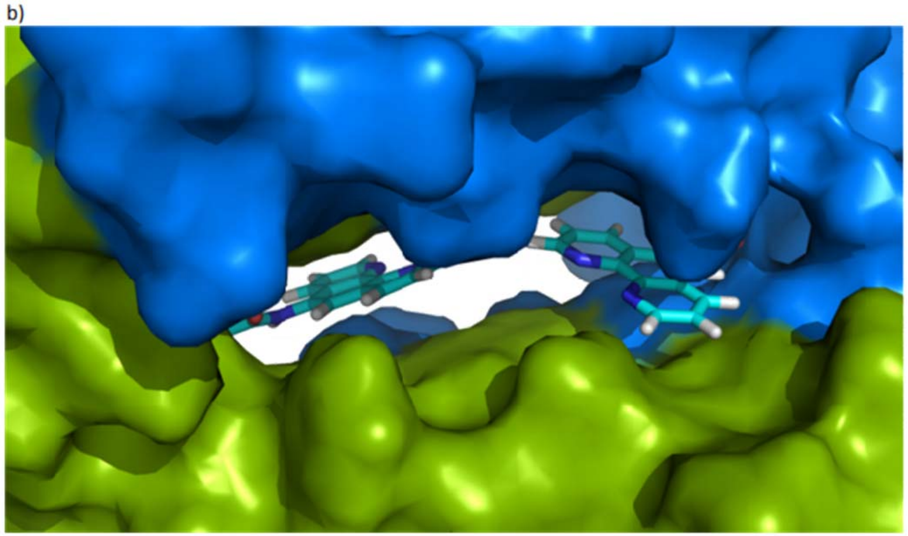


Entry	Catalyst	Conversion (%)	Endo/exo	ee (endo, %)
1	LmrR_N19C_**I**_Cu^{II}	24±3	92:8	53±5
2	LmrR_M89C_**I**_Cu^{II}	94±4	95:5	97±1
3	LmrR_M89C_V15A_**I**_Cu^{II}	89±4	96:4	97±1
4	LmrR_M89C_D100E_**I**_Cu^{II}	38±4	88:12	40±2
5	LmrR_M89C_Cu^{II}	30±5	90:10	<5
6	LmrR + Phenanthroline + Cu^{II}	29±8	90:10	28±7
7	Phenanthroline + Cu^{II}	20±5	93:7	0

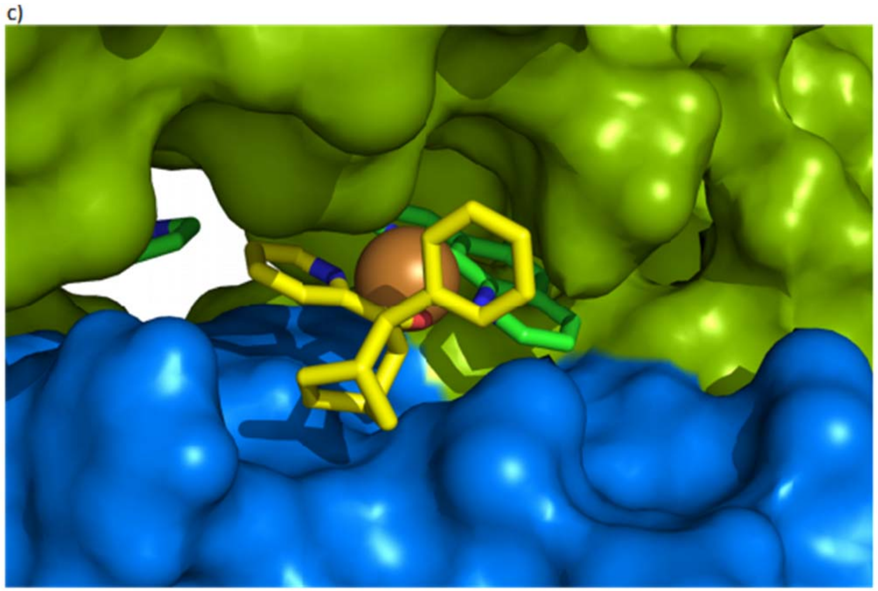
Mechanisms



a) Cartoon representation.



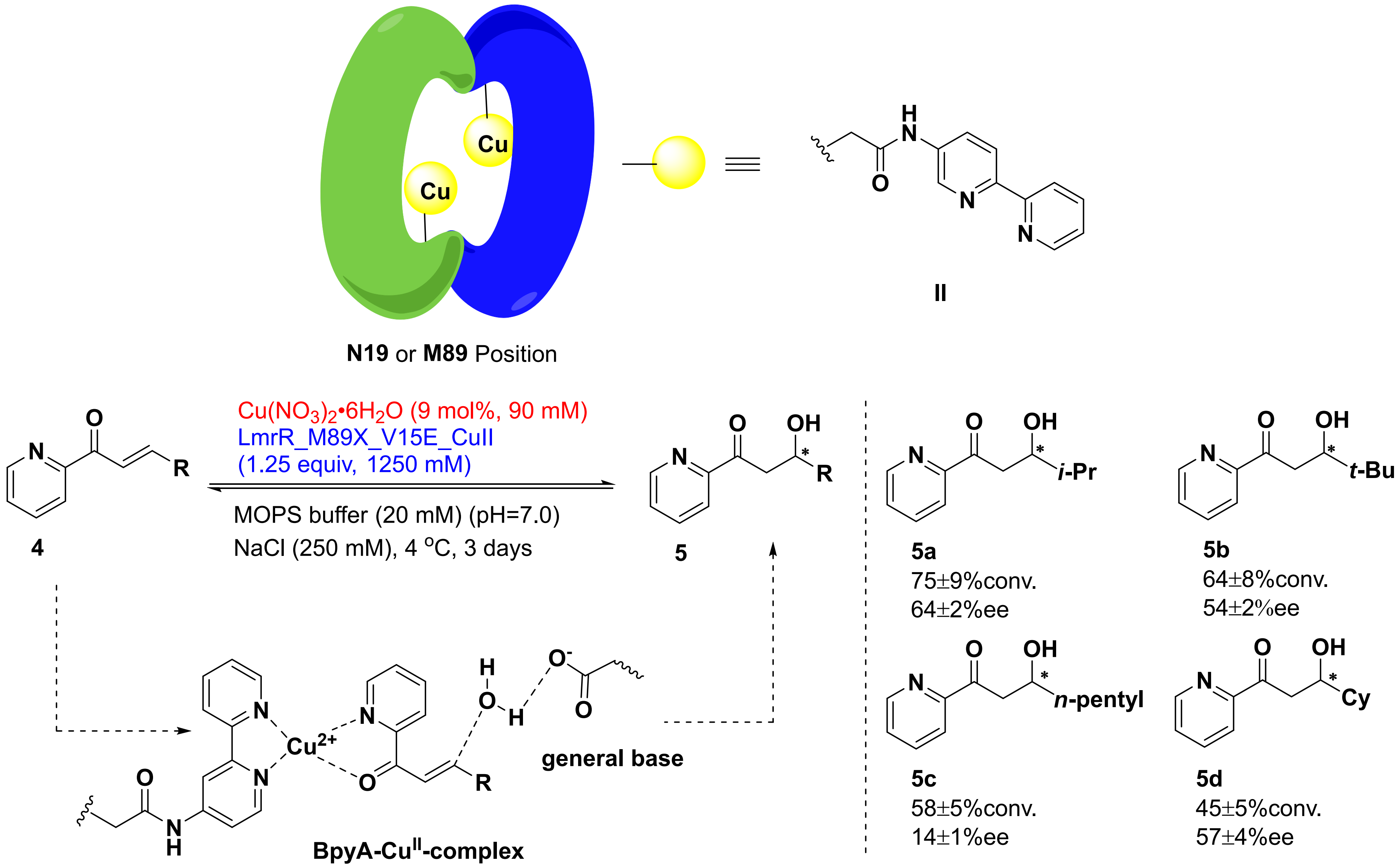
b) Space filled representation.



c) Manual docking of Cu(II) bound product **3** inside the LmrR pocket.

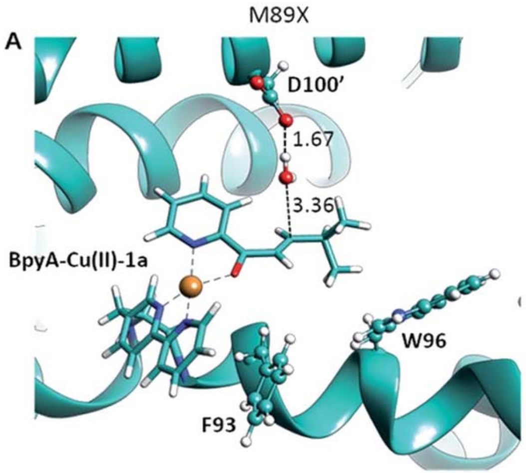
Protein scaffold is a source of chirality.
The reaction is protein accelerated.
The conversion and ee values depend on the ligand.
The newly created active site in the hydrophobic pocket.

The Copper Enzyme Catalyzed Hydration reactions

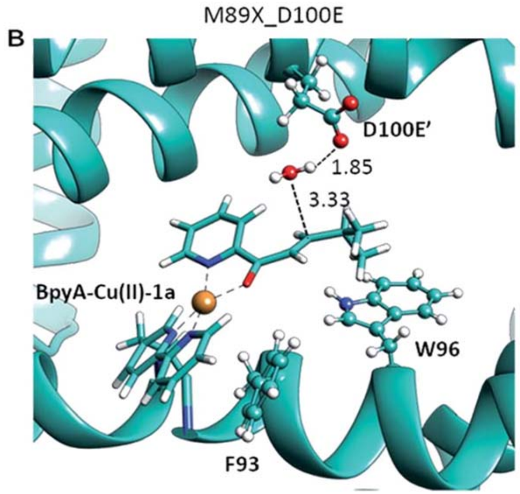


Drienovska, I.; Alonso-Cotchico, L.; Vidossich, P.; Lledos, A.; Marechal, J-D.; Roelfes, G. *Chem. Sci.* **2017**, *8*, 7228.

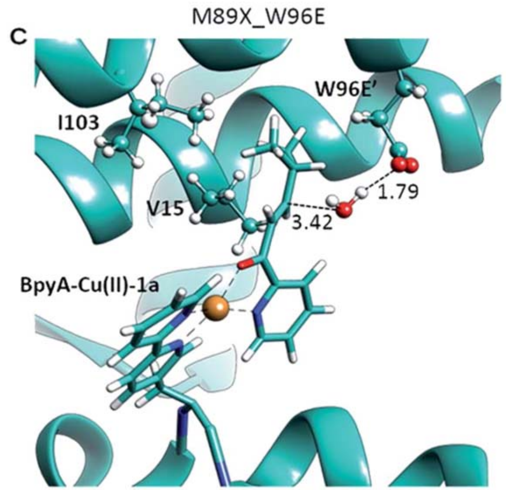
Mechanisms



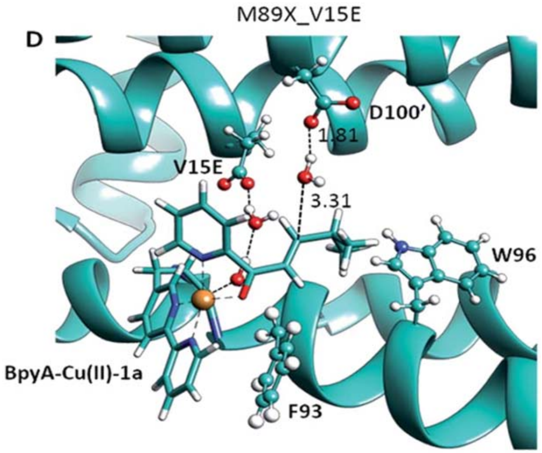
Water accessibility is not equivalent on the pro-chiral faces of the substrate and D100 was not to approach the double bond of the substrate closely enough.



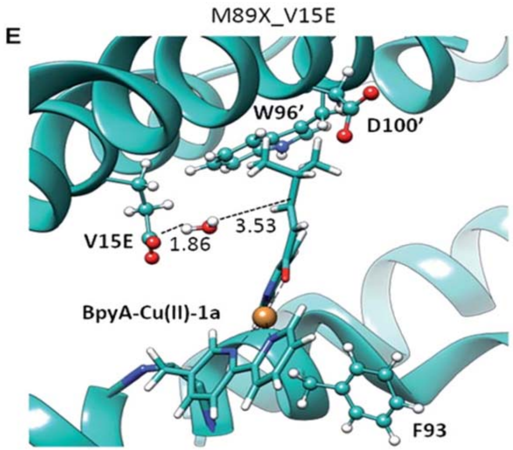
F93 is involved in π–π stacking with the bipyridine complex.



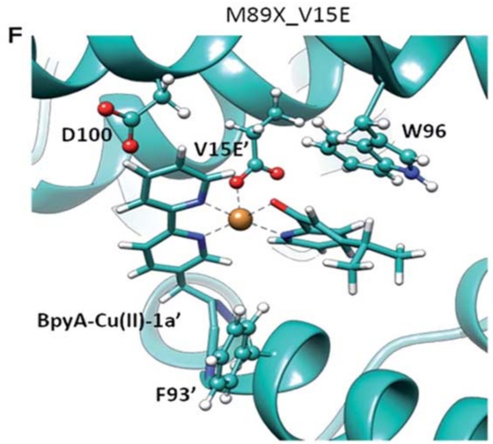
BpyA–Cu^{II}-complex stay at the dimer interface.



The product of R configuration has a larger contact area with water.

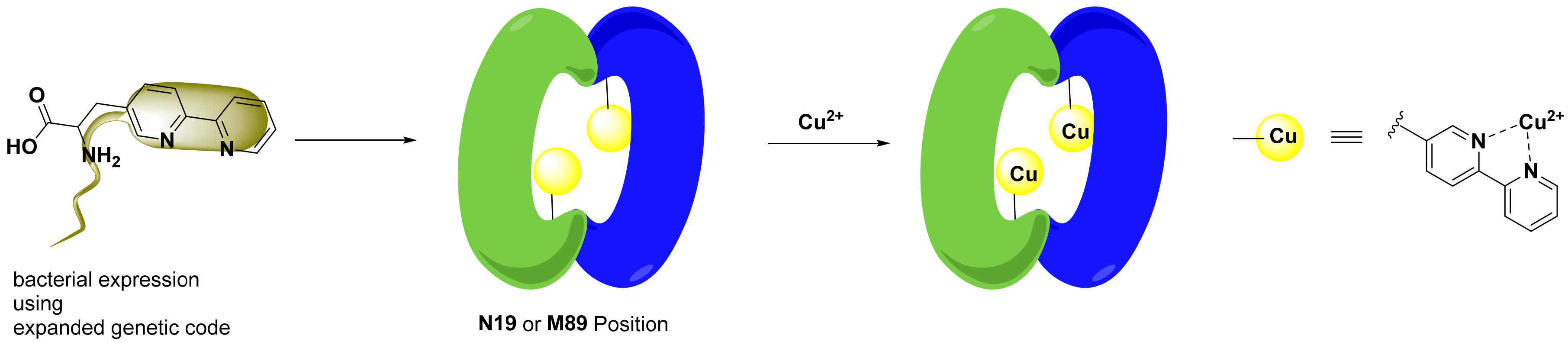


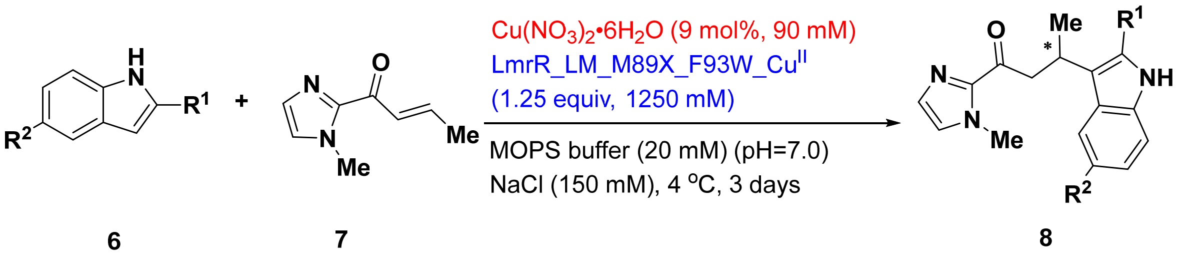
Activation of the H_2O nucleophile by E15 preferentially takes place at the pro-R face.

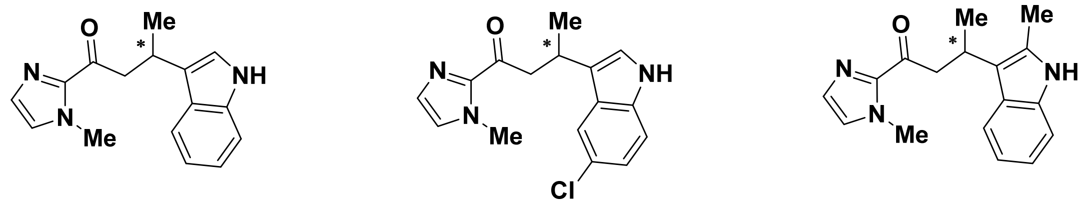


This stabilizing interaction is hinders the approach of water to the pro-S face of the substrate.

The Copper Enzyme Catalyzed Friedel-Crafts Alkylation



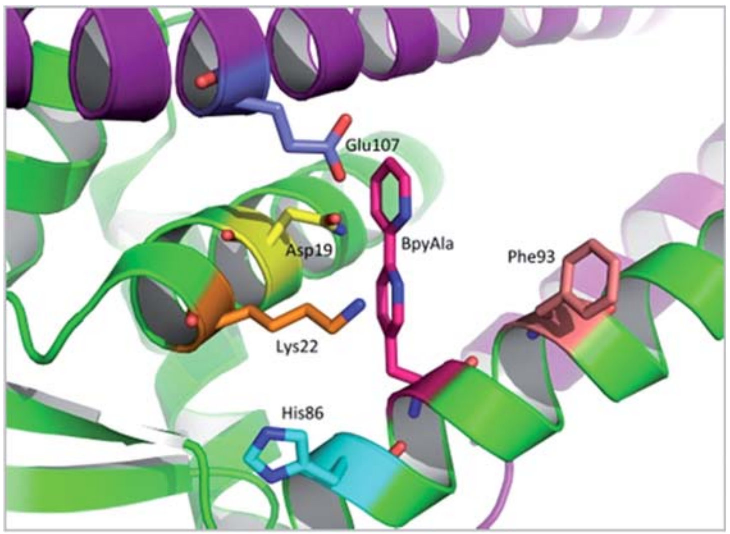




8ba 16±6%conv. 55±3%ee

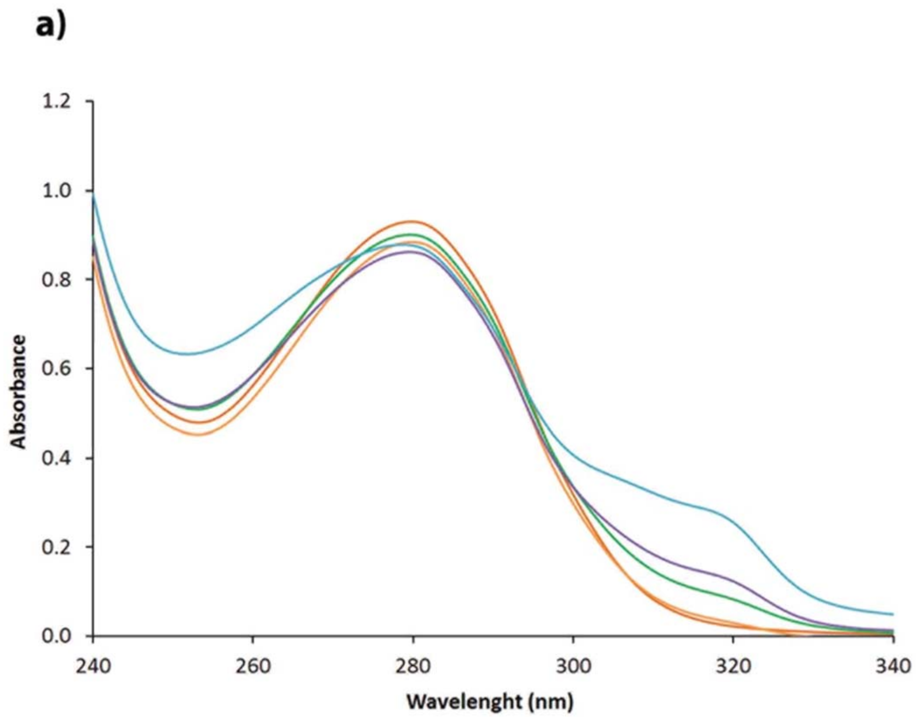
8ca 3±1%conv. 55±3%ee

8da 94±8%conv. 83±0%ee

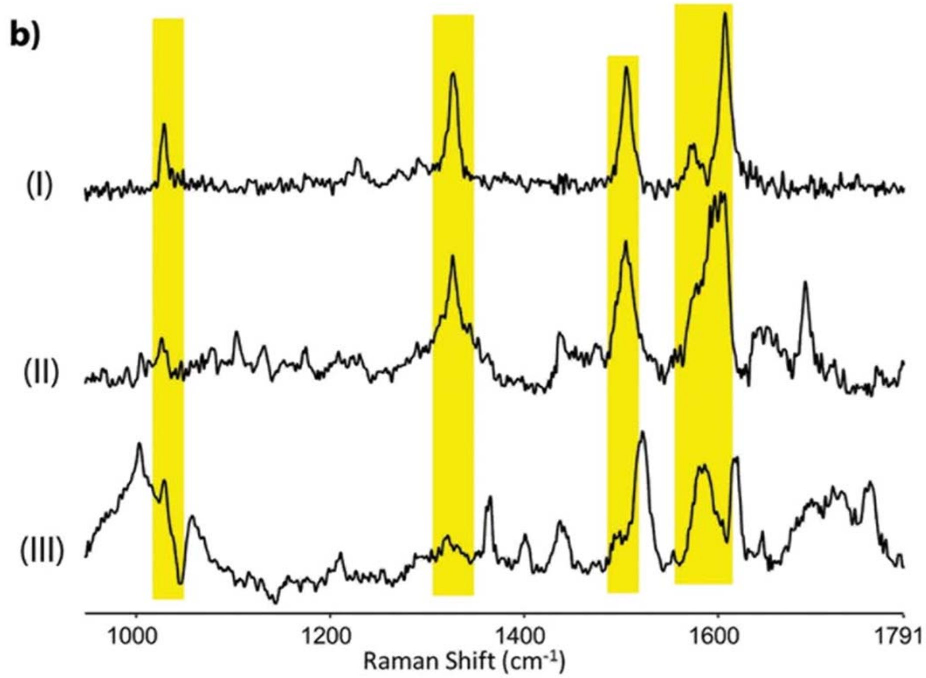


Drienovska, I.; Rioz-Martinez, A.; Draksharapu, A.; Roelfes, G. *Chem. Sci.* **2015**, *6*, 770.

Spectra study



(a) Absorption spectra of LmrR_M89BpyAla after addition of different concentrations of $Cu(NO_3)_2$.

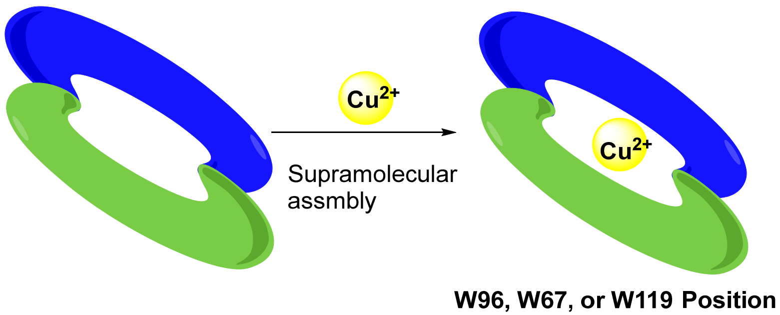


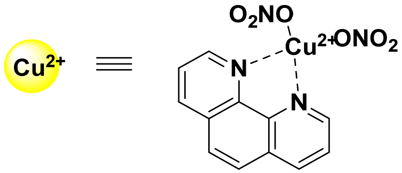
b) Raman spectra of (I) Cu^{II}-BpyAla,
(II) LmrR_M89BpyAla_Cu^{II},
(III) LmrR_M89BpyAla.

These UV/Vis absorption bands are attributed to the red shifted π–π* transition of the bipyridine moiety of the incorporated BpyAla upon binding of Cu(II).

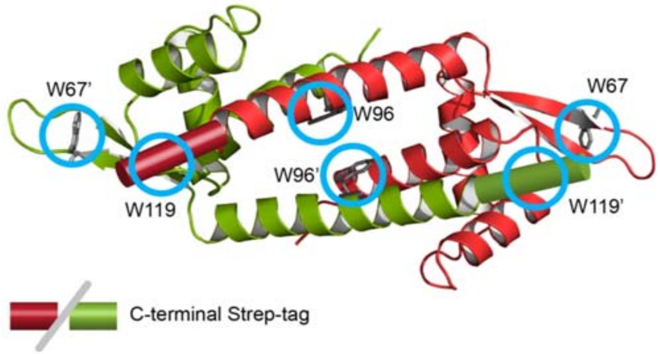
The Raman spectra bands are typical of pyridyl based ligands complexed to metal ions.

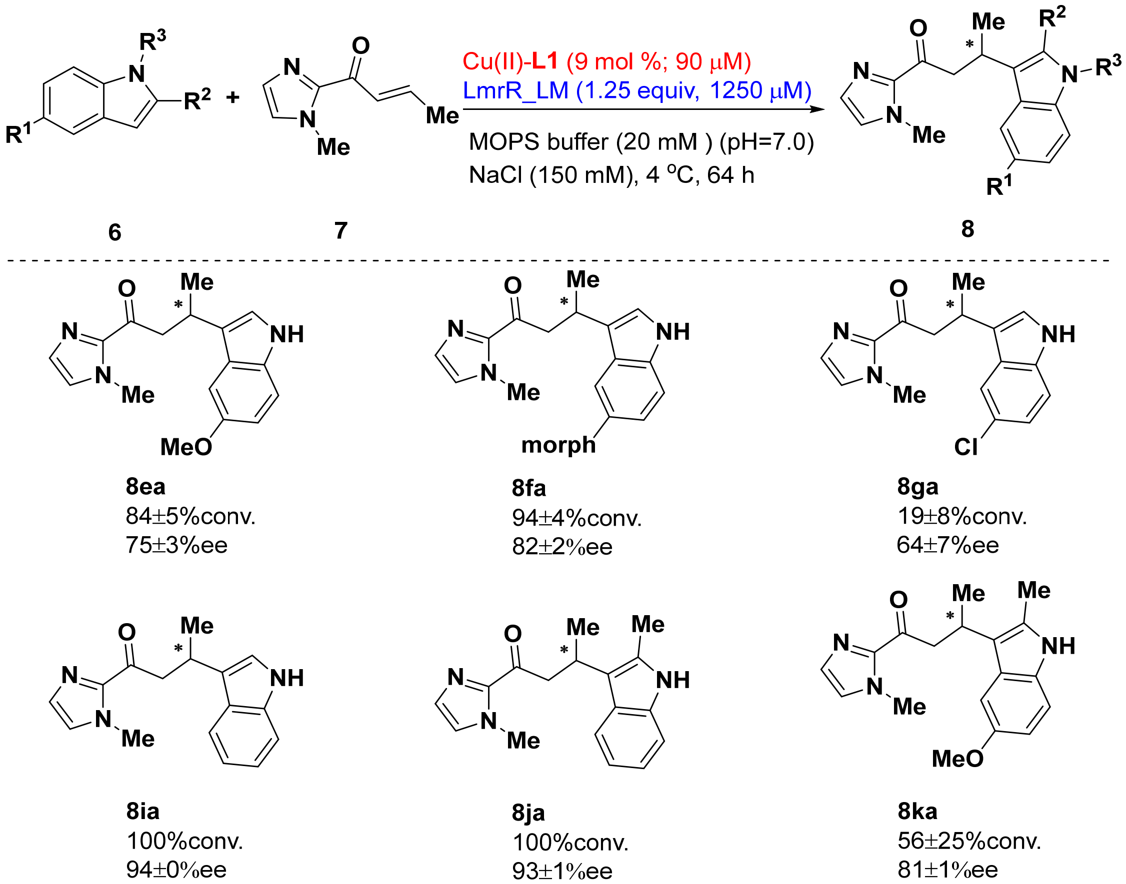
The Copper Enzyme Catalyzed Friedel-Crafts Alkylation





Cu(II)-**L1**

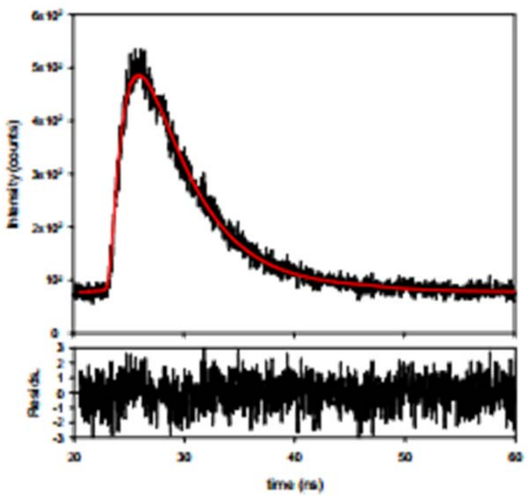




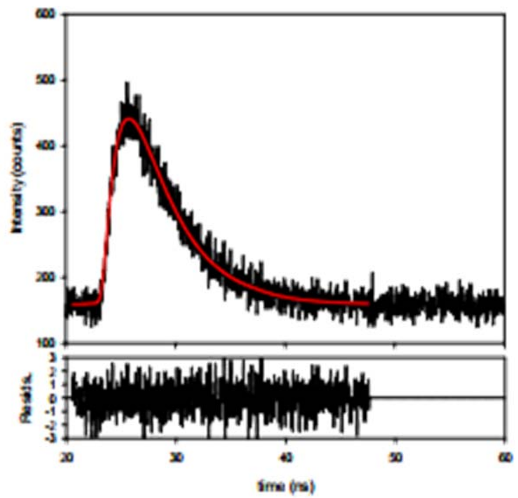
Bos, J.; Browne, W. R.; Driessen, A. J. M.; Roelfes, G. *J. Am. Chem. Soc.* **2015**, *137*, 9796.

Fluorescence decay lifetime experiments

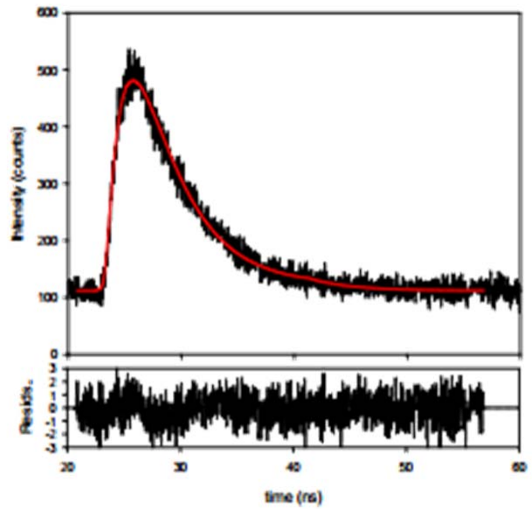
LmrR_LM



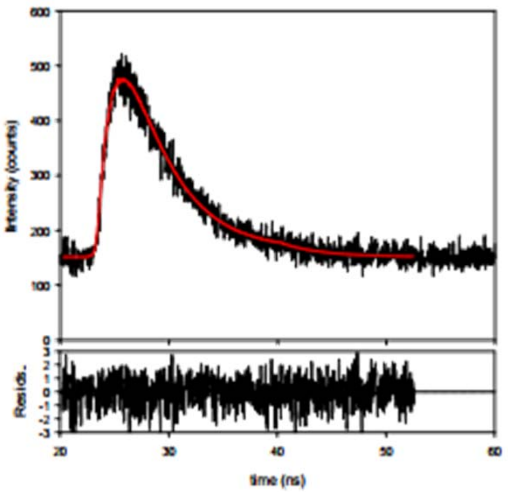
LmrR_LM_Cu(II)-**L1** (44μM)



LmrR_LM_W96A



LmrR_LM_W96A_Cu(II)-**L1** (44μM)

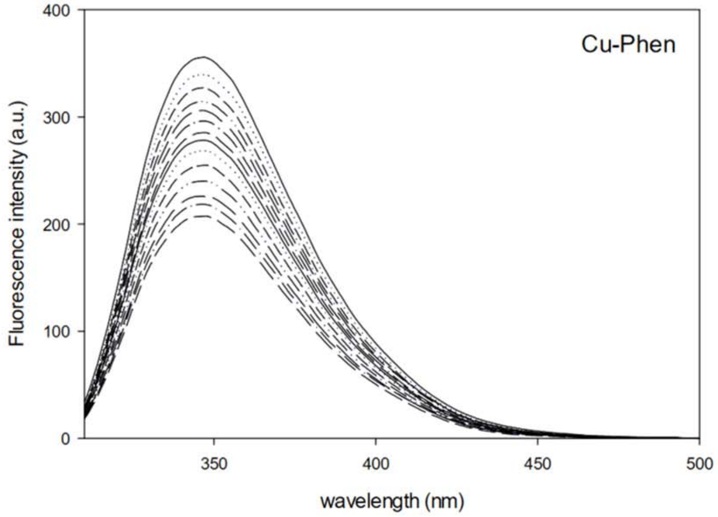


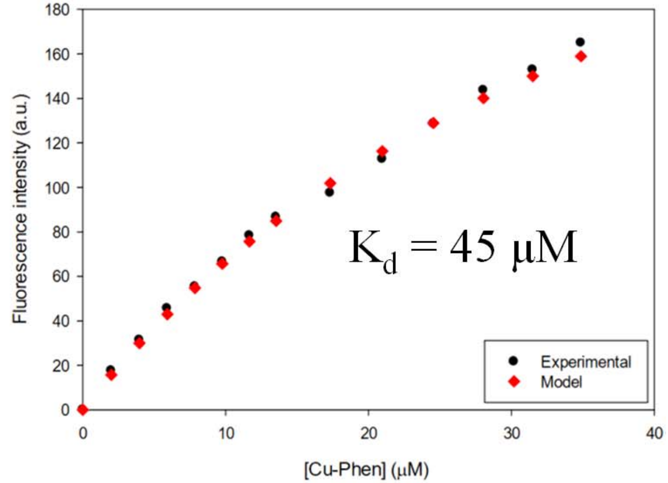
Protein	[Cu(II)-**L1**] (μM)	Lifetime (ns)
LmrR_LM	0	4.4
LmrR_LM	44	3.5
LmrR_LM_W96A	0	3.7
LmrR_LM_W96A	44	3.7

These data indicate that Cu(II)-complex binds predominantly in proximity to W96/W96′, which suggests it is bound at or near to the hydrophobic pore of LmrR.

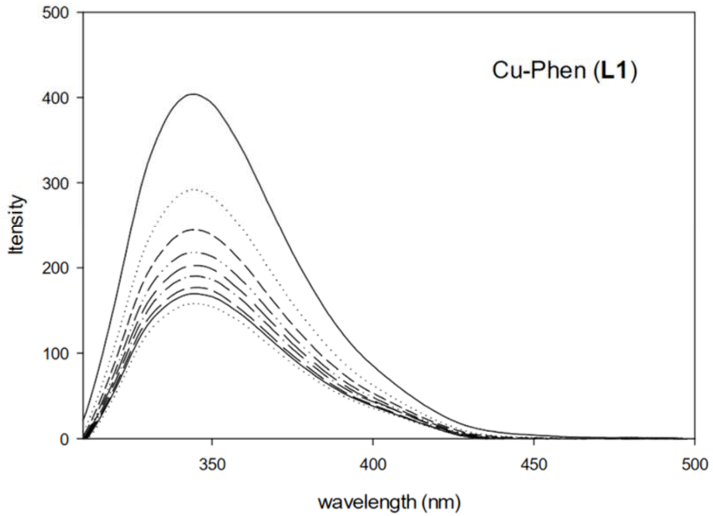
Fluorescence titration experiments

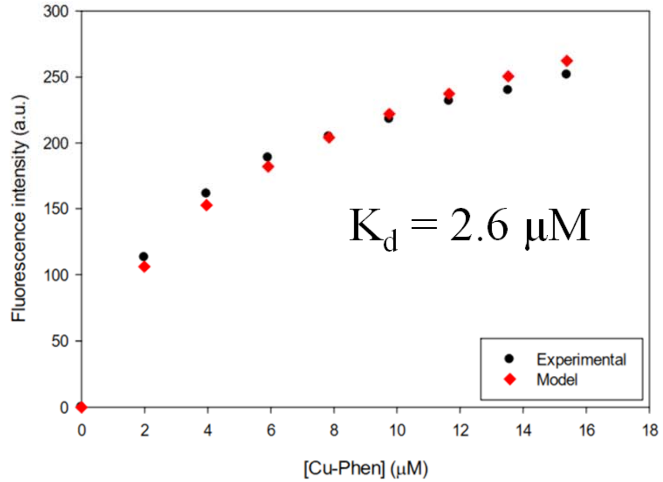
LmrR_LM_W96A + Cu(II)-**L1** (Cu-Phen)





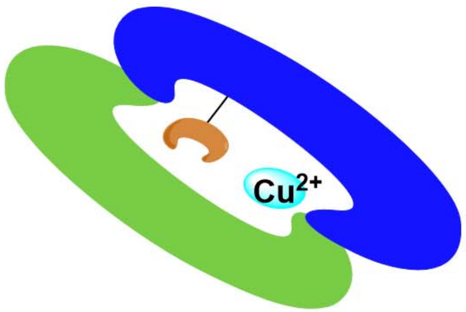
LmrR_LM + Cu(II)-**L1** (Cu-Phen)



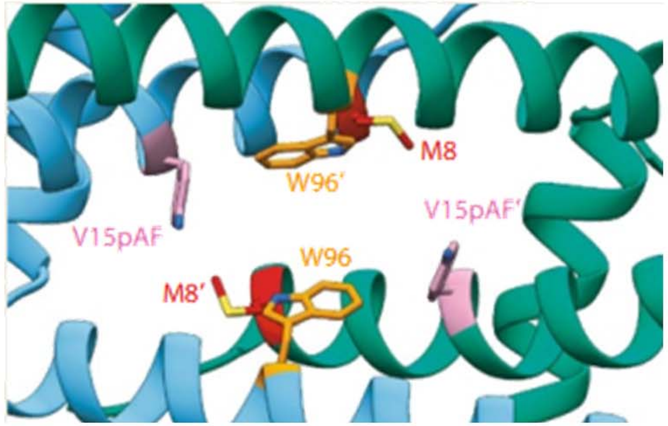


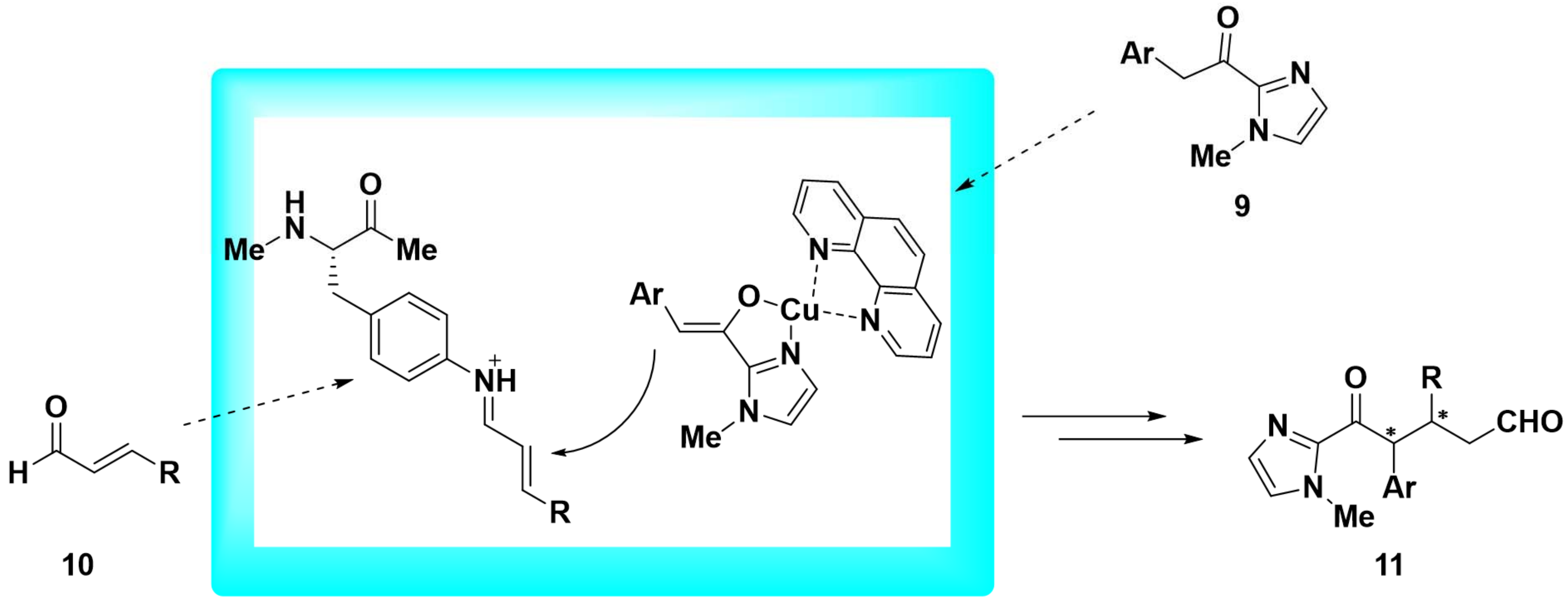
These data indicate that LmrR_LM_W96A and substrate are more matched, so the response is faster.

The Copper Enzyme Catalyzed Michael Addition



V15 Position

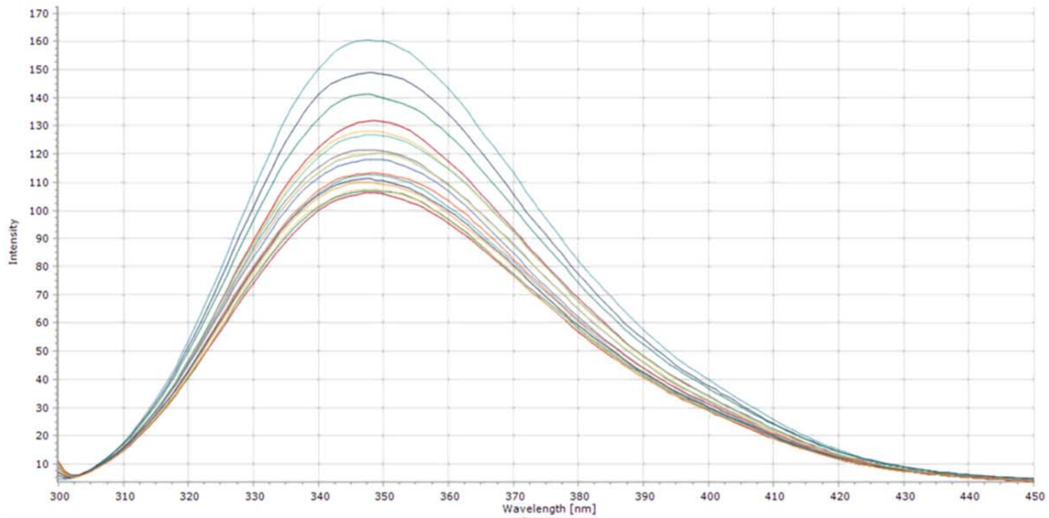


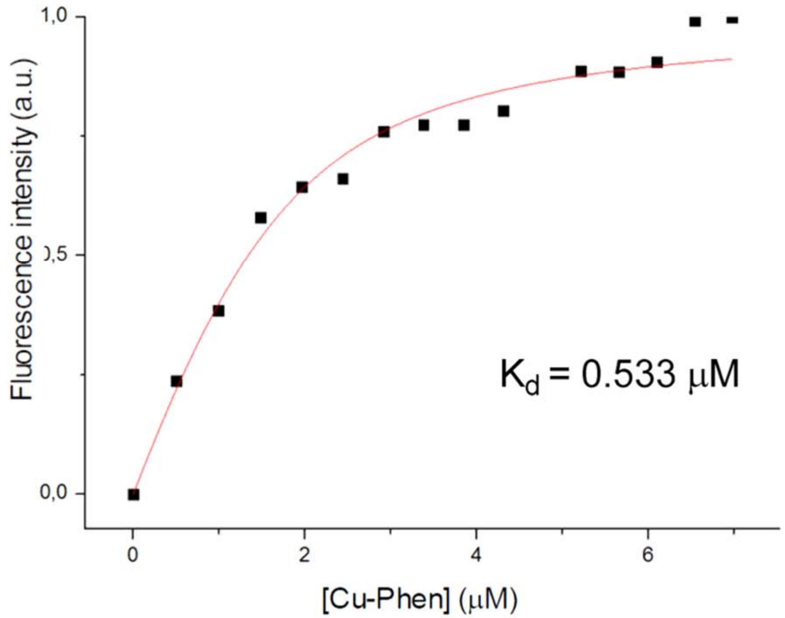


Zhou, Z.; Roelfes, G. *Nat. Catal.* **2020**, *3*, 289.

Fluorescence titration experiments

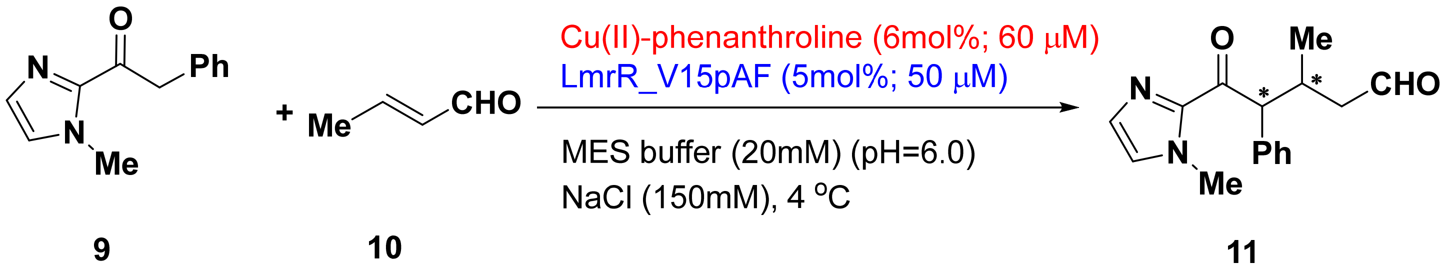
LmrR_V15pAF + Cu(II)-phenanthroline

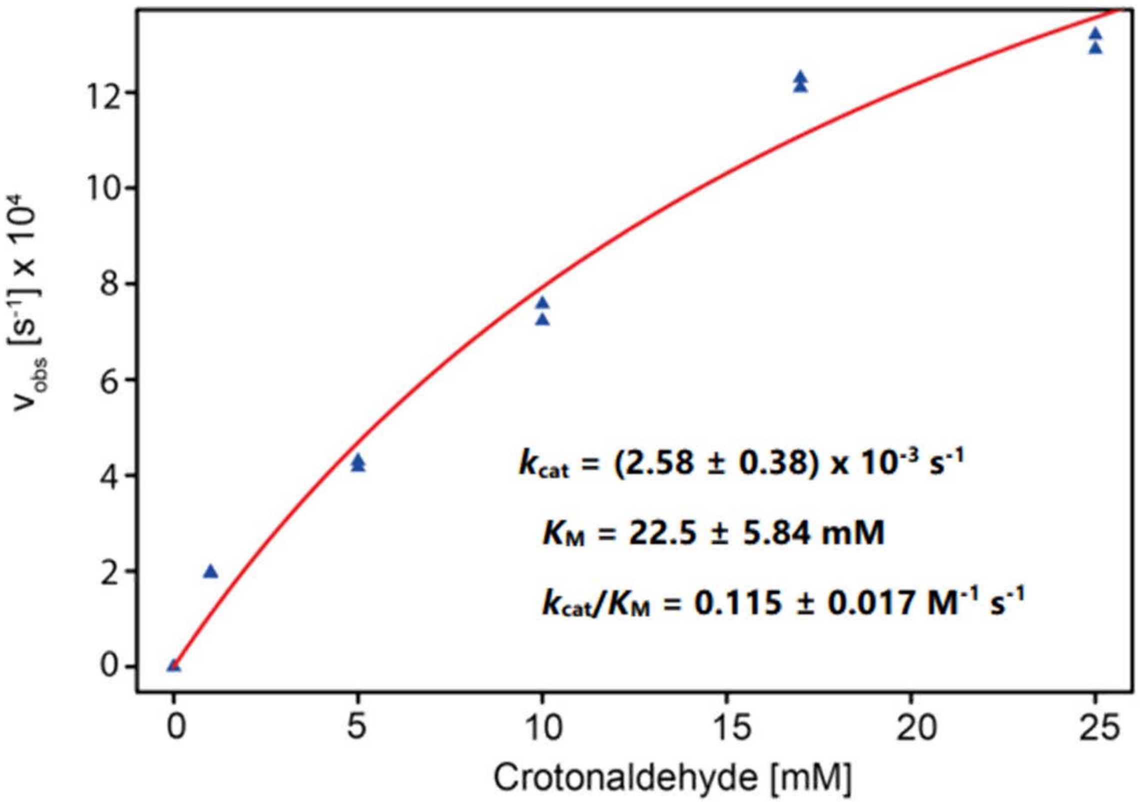




These data indicate that the pAF residue does not negatively affect the binding of Cu(II)-complex to the two tryptophan residues (W96/W96').

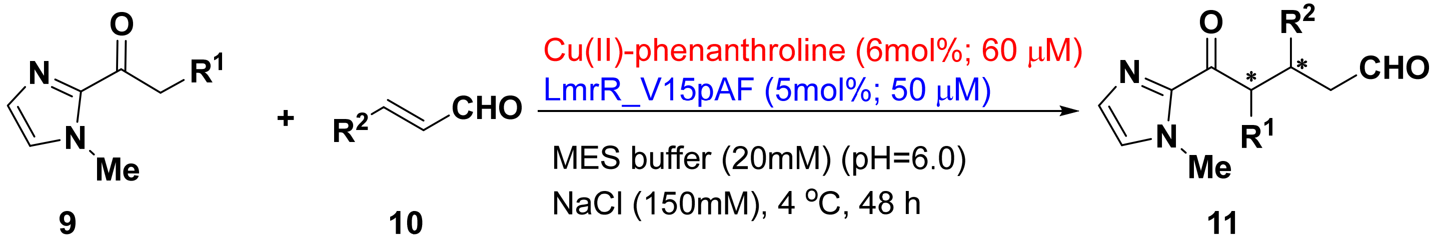
Kinetic experiments





Michaelis-Menten equation

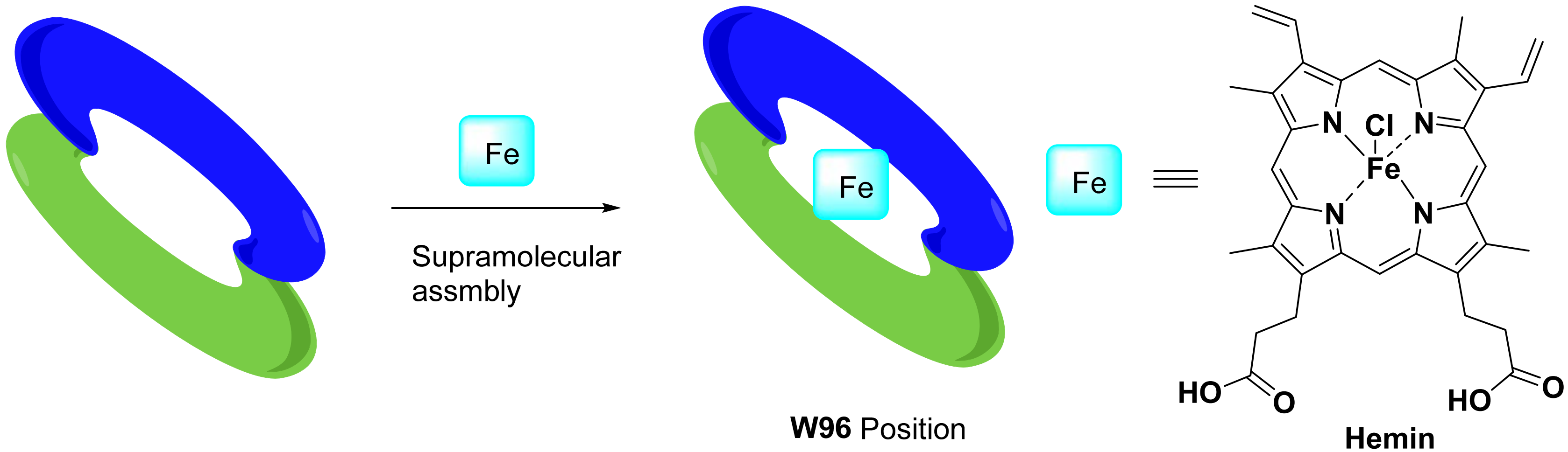
Scope Study

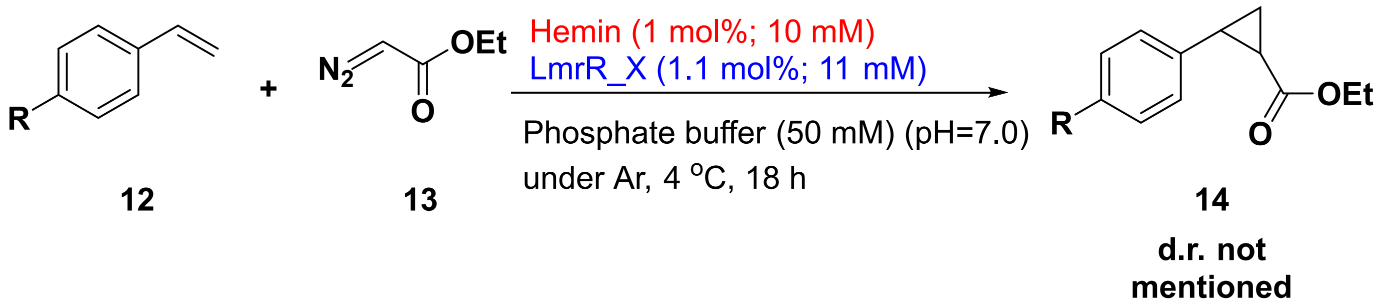


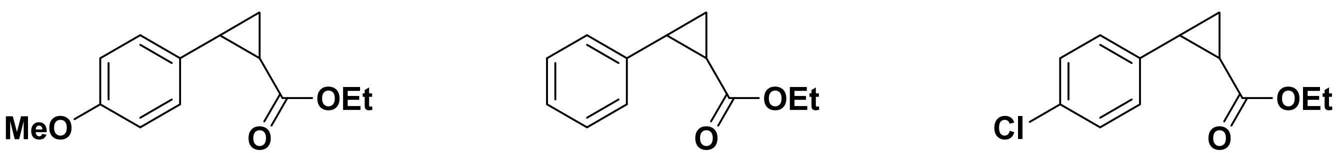
a R^1 = Ph; R^2 = H.
b R^1 = Ph; R^2 = Me.
c R^1 = Ph; R^2 = *n*-Pr.
d R^1 = Ph; R^2 = Ph.
e R^1 = *p*-MeOPh; R^2 = Me.
f R^1 = *p*-MeOPh; R^2 = H.
g R^1 = *p*-ClPh; R^2 = Me.
h R^1 = 3-Thienyl; R^2 = Me.

Entry	Product	LmrR_V15pAF+Cu(II)-phen			LmrR_V15pAF_M8L+Cu(II)-phen		
		Yield (%)	d.r.	ee(%)	Yield (%)	d.r.	ee(%)
1	**11a**	42±3	-	85±2	35±2	-	85±1
2	**11b**	65±1	4:1	98±0/86±1	82±1	6:1	>99±0/93±1
3	**11c**	32±3	4:1	98±0/82±1	48±2	5:1	97±0/85±1
4	**11d**	56±6	2:1	61±5/18±2	52±4	2:1	72±3/12±2
5	**11e**	72±3	8:1	99±0/85±1	90±2	9:1	>99±0/85±1
6	**11f**	53±2	-	96±1	55±3	-	97±0
7	**11g**	80±2	7:1	97±0/67±1	88±1	8:1	98±0/80±1
8	**11h**	46±3	5:1	98±0/72±2	82±2	6:1	>99±0/81±1

The Iron Enzyme Catalyzed Cyclopropanation reaction



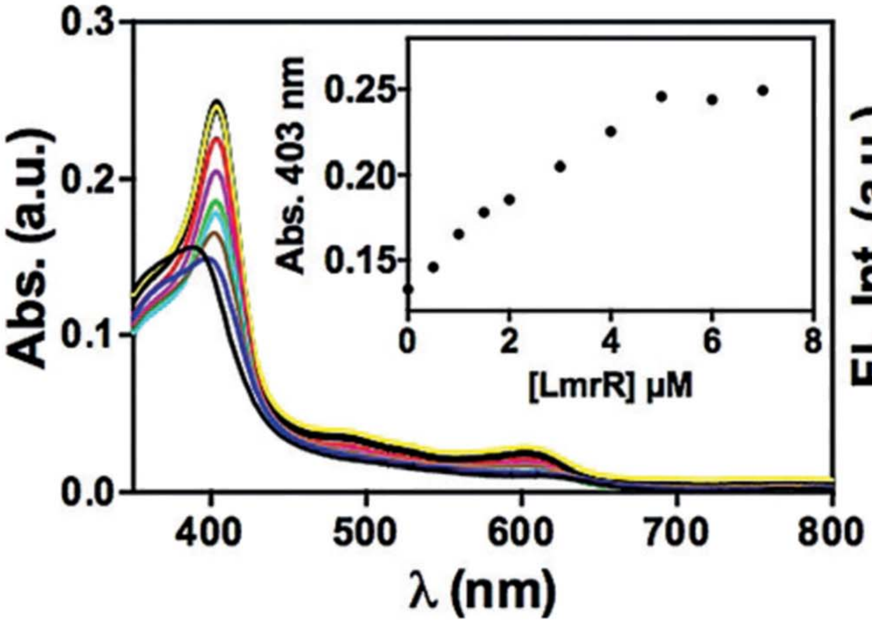


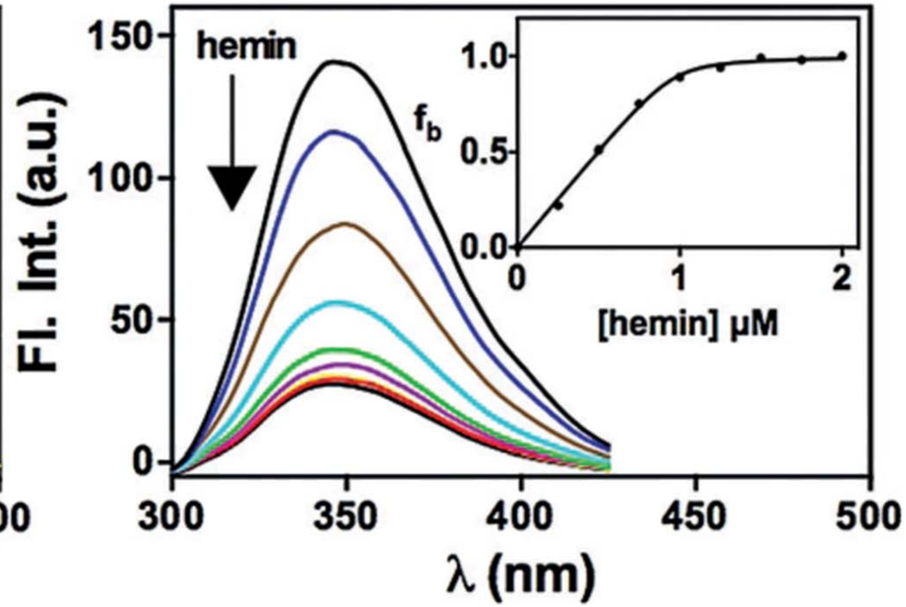


14a	**14b**	**14c**
45±9%conv.	39±13%conv.	35±13%conv.
51±14%ee	38±5%ee	25±5%ee

Villarino, L.; Splan, K. E.; Reddem, E.; Alonso-Cotchico, L.; Souza, C. G.; Lledos, A.; Marechal, J-D.; Thunnissen, A-M. W. H.; Roelfes, G. *Angew. Chem. Int. Ed.* **2018**, *57*, 7785.

Spectra study





$K_d = 38 \pm 27$ nM

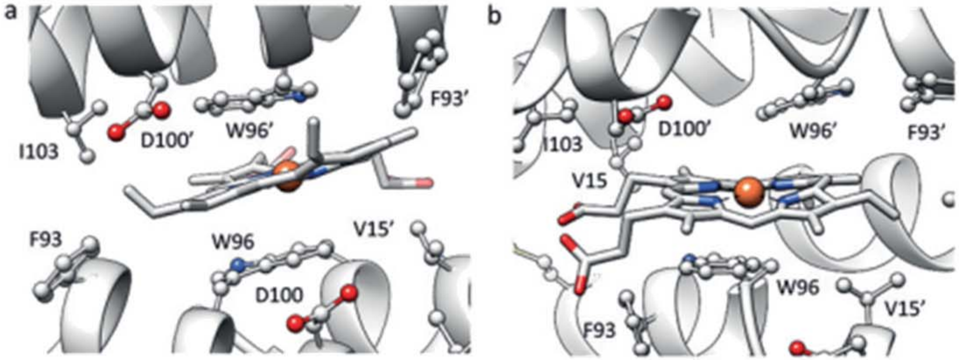
a) Electronic absorption spectra upon the addition of LmrR to 5 μm hemin.

b) Fluorescence spectra upon the addition of hemin to 1 μm LmrR dimer.

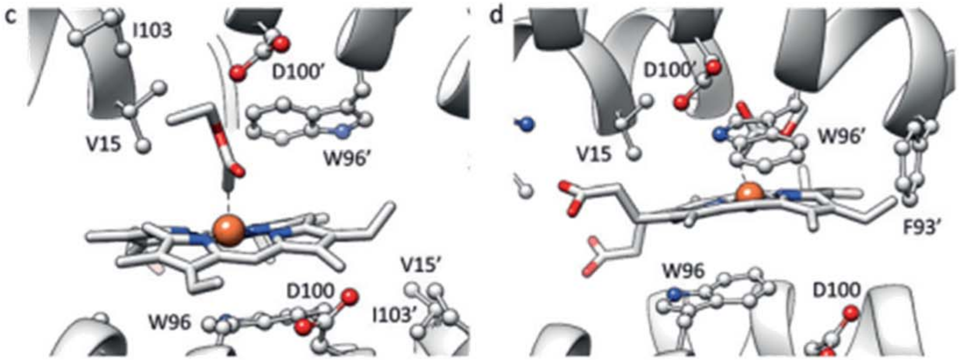
Upon addition of the protein, the dimeric porphyrin structure is disrupted and the hemin is in a monomeric form.
Heme: protein = 1:1.

The fluorescence titration data indicating that the affinity of the protein for hemin is quite high.

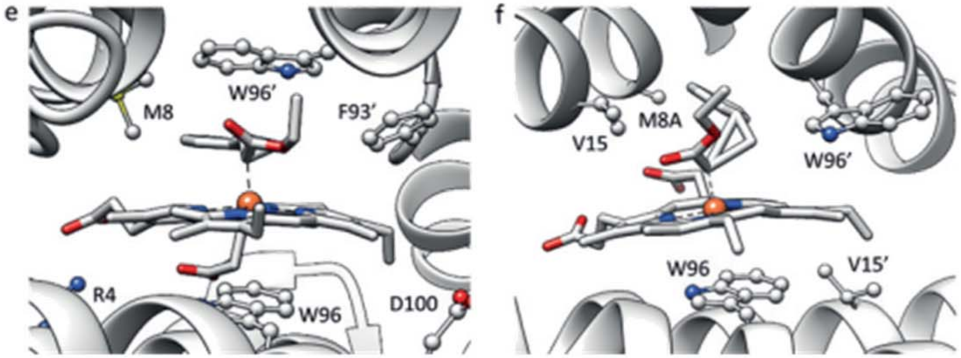
Mechanism



a, b) The LmrR-heme system.

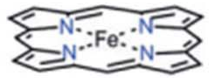


c, d) The LmrR–heme–carbine.



e, f) The cyclopropanation transition state.

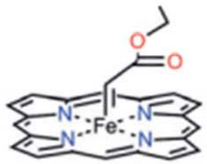
5



a) The iron atom cannot be accessible to solvent.

b) The iron site becomes accessible to bind the carbine.

6



c) With W96’ pointing towards the hydrophobic core, carbe-ne will be accessible for the styrene co-substrate.

d) Rotation of W96’ to the outside of the pore causes the heme–carbene (**6**) to remain at the dimer interface.

7

e) The dimer interface become broader to accommodate the catalytic complex.

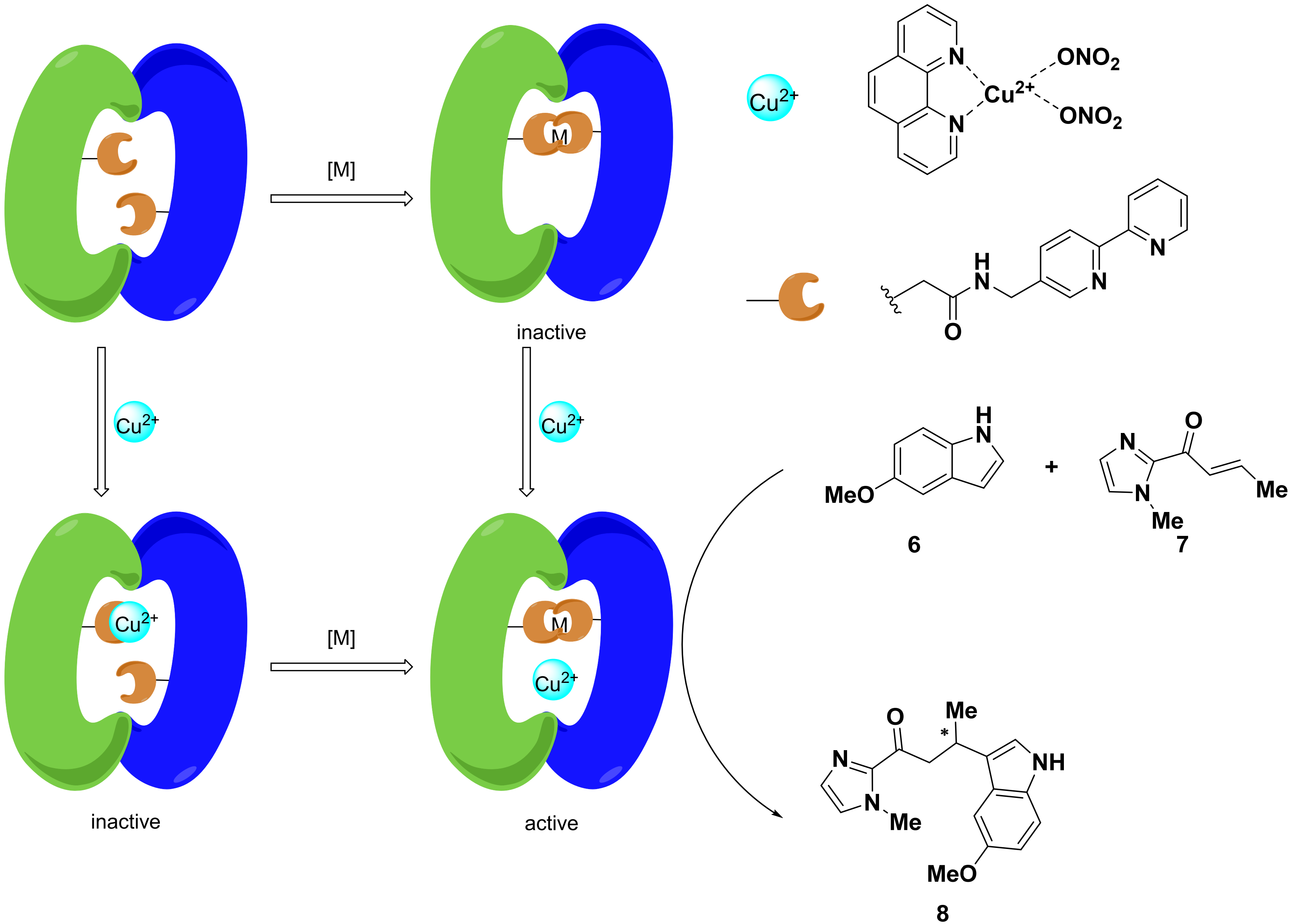
f) W96 is flipped towards the solvent and thus contributes to binding of the TS (**7**) structure into the dimer interface.

1. Background

2. ArM Catalyzed Asymmetric Organic reactions
   - The Copper Enzyme Catalyzed reactions
   - The Iron Enzyme Catalyzed reactions
   - The Bimetallic Enzyme Catalyzed reactions

3. Summary and outlook

The Bimetallic Enzyme Catalyzed Friedel-Crafts Alkylation

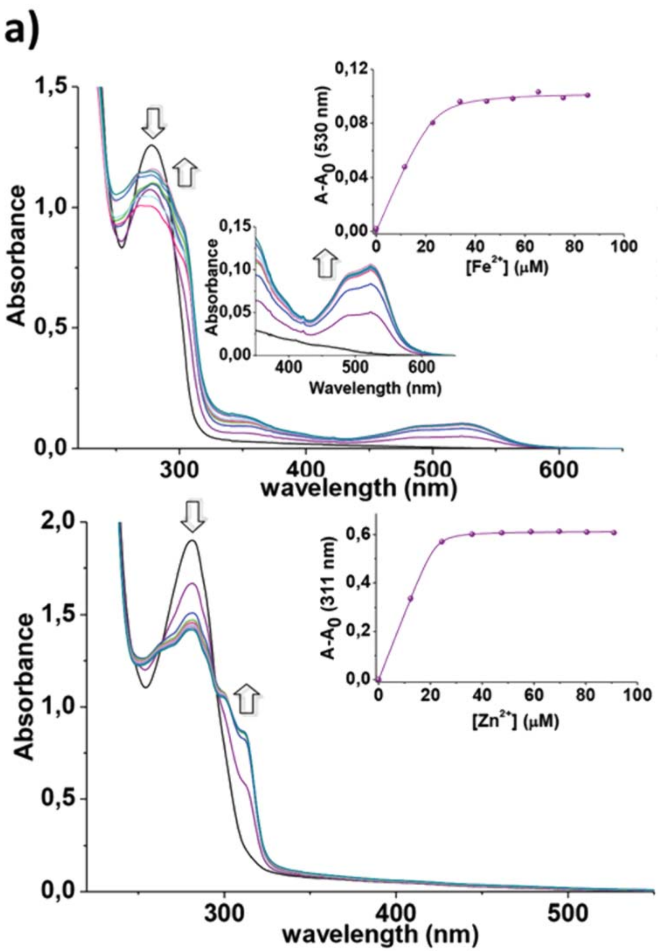


Bersellini, M.; Roelfes, G. *Dalton Trans.* **2017**, *46*, 4325.

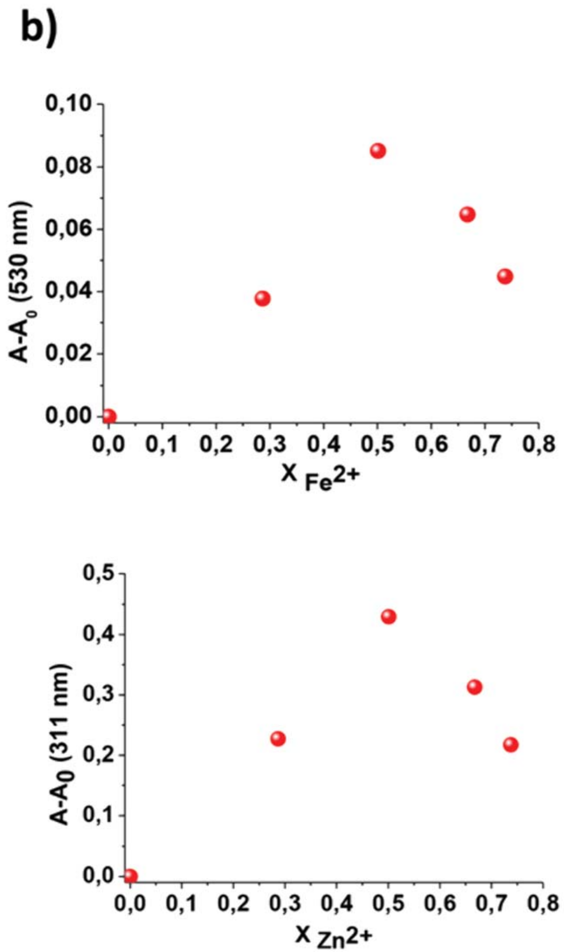
Spectra study

$FeSO_4 \cdot 7H_2O$

$Zn(NO_3)_2 \cdot 6H_2O$



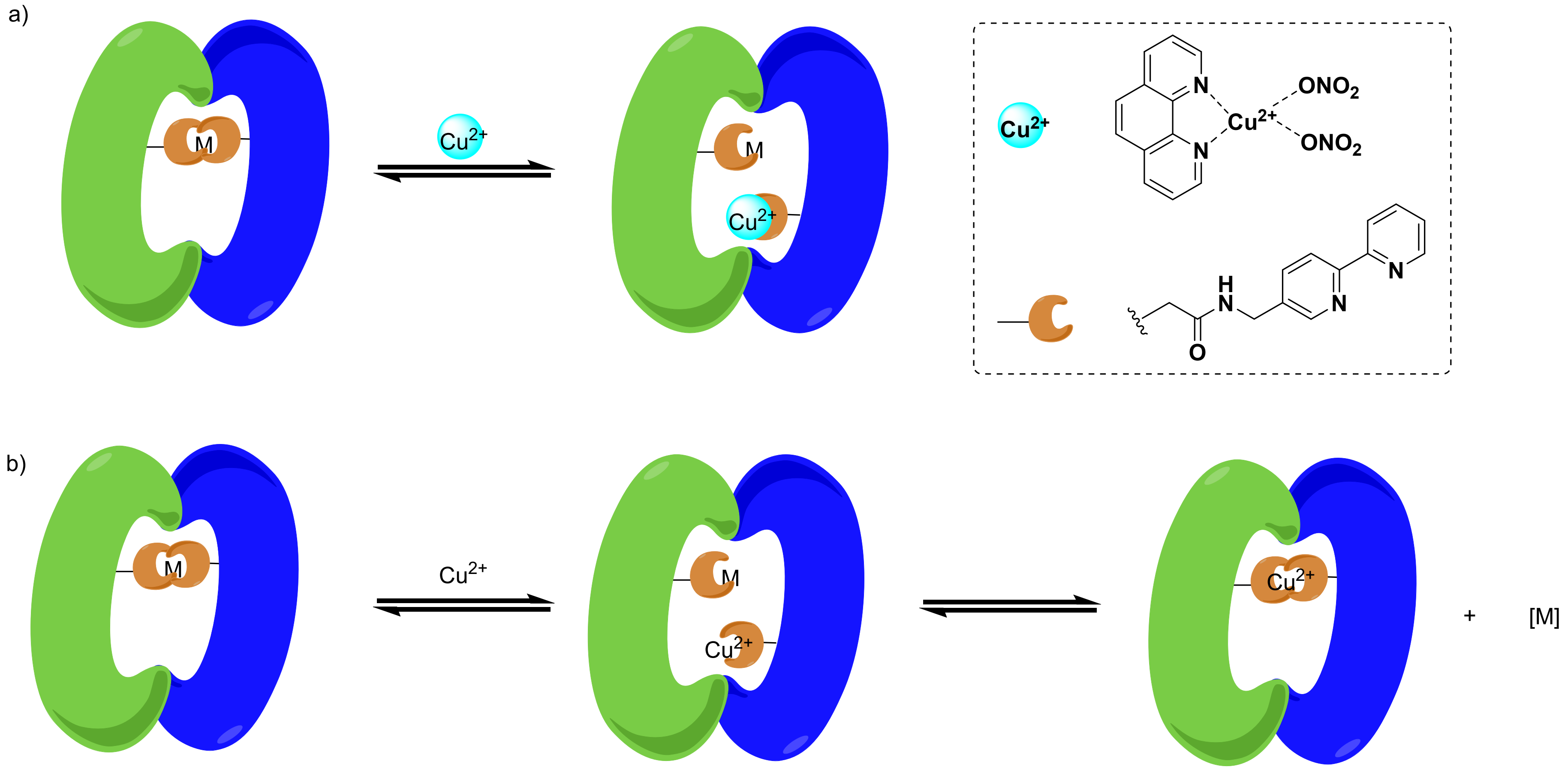
a) UV-visible titrations of LmrR E104C_bpy with metal salt.



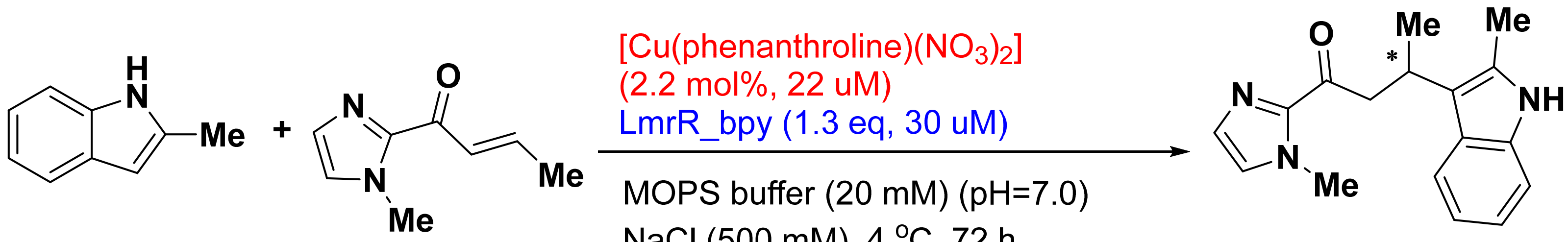
b) Job's plot graphs of LmrR E104C_bpy with metal salt.

Upon addition of either metal ion, the appearance of a shoulder around 310 nm was observed, indicative of a change in the π–π* transition of the bipyridine upon metal coordination.
The absorption bands between 490 and 530 nm are characteristic of Fe^{2+} bipyridine complexes.
Metal ion: protein = 1:1.

Control experiments



Control experiments



6 7 8

Entry	1st incubation	2nd incubation	Yield (%)	TON	ee (%)
1	$[Cu(phen)(NO_3)_2]$	-	<1	<1	n.d.
2	$FeSO_4 \cdot 7H_2O$	$[Cu(phen)(NO_3)_2]$	57±14	23±5	72±14
3	$[Cu(phen)(NO_3)_2]$	$FeSO_4 \cdot 7H_2O$	33±5	14±2	78±2
4	$Zn(NO_3)_2 \cdot 6H_2O$	$[Cu(phen)(NO_3)_2]$	13±2	6±1	50±1
5	$[Cu(phen)(NO_3)_2]$	$Zn(NO_3)_2 \cdot 6H_2O$	11±3	5±1	50±13

This result suggests that selective regulation of activity of the designed artificial metalloenzyme by Zn^{2+} is reversible.

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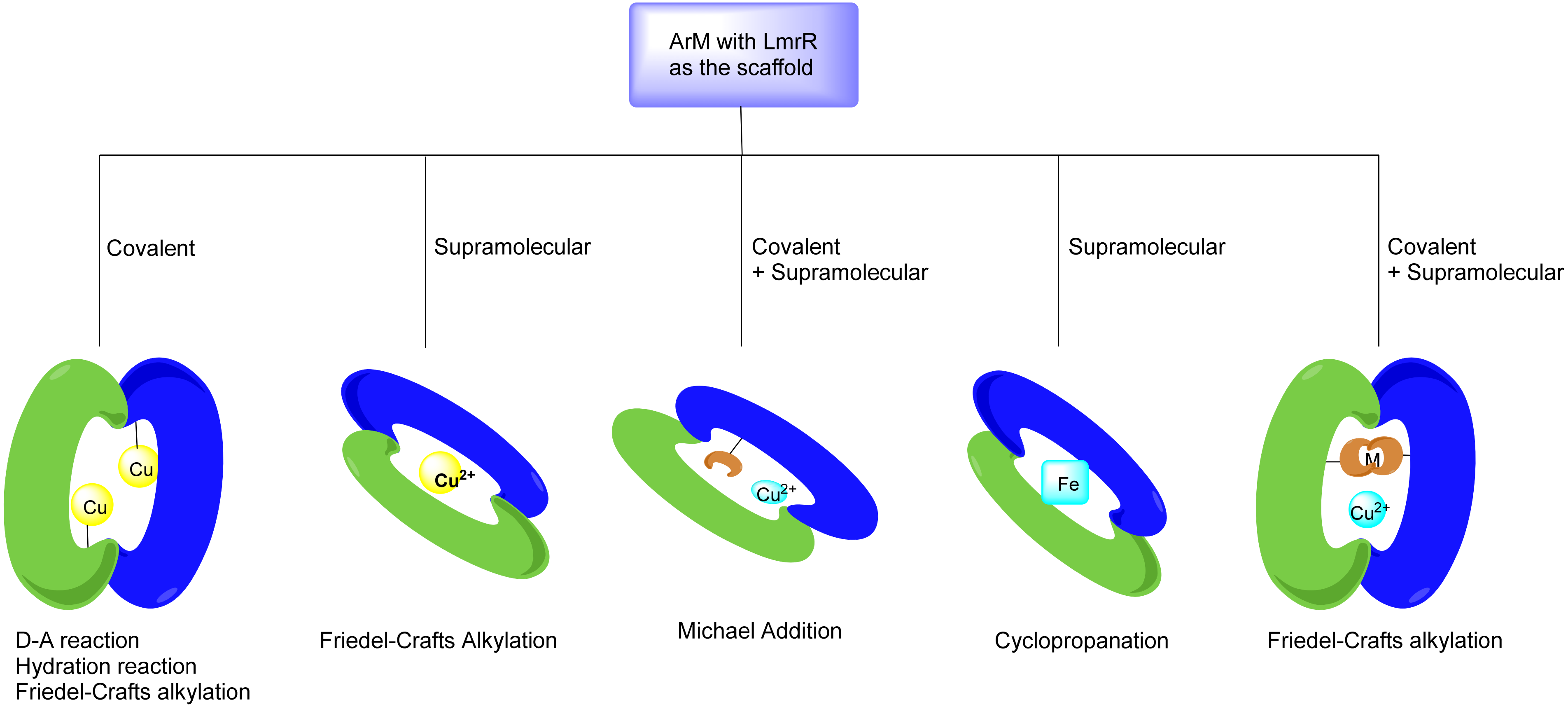
Summary



Advantages:

- High reactivity
- Mild reaction conditions
- Green catalysis
- Higher atomic utilization

Reaction mode:

- Reduction Chemistry
- C-C Bond Formation
- Oxygen Insertion Chemistry
- Hydration

Disadvantages:

- High catalyst loading
- Unclear mechanism
- Long reaction time
- Narrow substrate scopes
- More additives

Outlook

- Improvements in the practicality of ArM catalysis could be realized using different immobilization procedures.

- Develop such transformations that can be accomplished using small molecule or enzyme catalysts.

- Added complexity of ArM formation relative to other systems might be offset by savings in other aspects of a process.

- ArM catalysis in vivo could ultimately be used to augment metabolic pathways with synthetic reactions to expand the scope of biosynthesis.

- ArM catalysis and other areas of protein engineering and organometallic chemistry.

Thanks for your attention!