The Yang Research Group
Precise Synthesis Lab at Tongji University

Gerard Roelfes Group's Work: Modification and Application of Artificial Enzymes

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Contents

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2000	the University of Groningen, the Netherlands	MSc and PhD	Advisor: Prof. Ben L. Feringa
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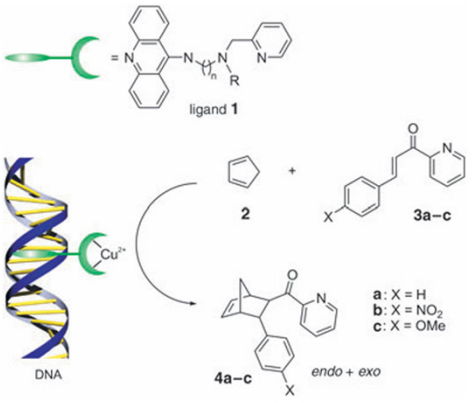
2003	the University of Groningen, the Netherlands	junior research group leader	
2006	the University of Groningen, the Netherlands	Assistant Professor	
2010	the University of Groningen, the Netherlands	Associate Professor	
2015	the University of Groningen, the Netherlands	Full Professor	

Research Areas

bio-inspired catalysis and chemical biology



Angew. Chem. Int. Ed. **2005**, *44*, 3230–3232

Table 1: Results of the catalytic Diels–Alder reaction with 1-naphthylmethyl- and 3,5-dimethoxybenzyl-substituted ligand **1**.[a]

Entry	Ligand	Ligand 1 R	*n*	Dienophile	Diels–Alder Product 4 *endo*/*exo*	*endo* [% *ee*]	*exo* [% *ee*]
1	**1a**		3	**3a**	98:2	49	18
2[b]	**1a**		3	**3a**	97:3	49	23
3[c]	**1a**		3	**3a**	98:2	47	23
4	**1a**		3	**3b**	96:4	37	16
5	**1a**		3	**3c**	98:2	48	24
6	**1b**		4	**3a**	98:2	33	19
7	**1c**		5	**3a**	97:3	<5	<5
8	**1d**		2	**3a**	96:4	−48	−37
9	**1e**		3	**3a**	98:2	−37	−7
10	**1f**		2	**3a**	92:8	−37	−78
11[b]	**1f**		2	**3a**	92:8	−34	−74
12[c]	**1f**		2	**3a**	92:8	−35	−82
13[d]	**1f**		2	**3a**	82:18	−34	−80
14	**1f**		2	**3b**	88:12	−47	−78
15	**1f**		2	**3c**	91:9	−53	−90

[a] All experiments were carried out with salmon testes DNA under the standard conditions (see Experimental Section) unless noted otherwise. [b] Conditions: catalyst (0.18 mM), dienophile (4 mM), cyclopentadiene (34 mM). [c] Calf thymus DNA. [d] DNA = synthetic duplex $d(GACT)_2$-$(AGTC)_2$ (0.39 mM), cyclopentadiene (21 mM), buffer contained NaCl (75 mM).

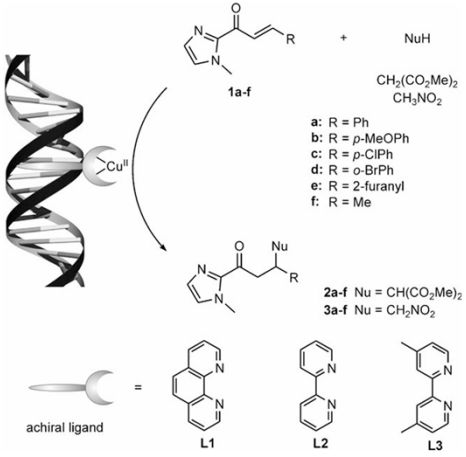


Table 1: Results of Michael addition reactions catalyzed by DNA/[Cu(L1–L3)$(NO_3)_2$].[a]

	[Cu(L*n*)$(NO_3)_2$]		Substrate		$CH_2(CO_2Me)_2$		CH_3NO_2	
	[mM]	L	R	equiv[b]	conv [%][c]	*ee* [%][d]	conv [%][c]	*ee* [%][d]
1	0.3	–	Ph (**1a**)	3.3	26	<2	0	n.d.[l]
2	0.3	L1		3.3	4	56 (–)	–	–
3	0.3	L2		3.3	90	80 (–)	–	–
4	0.3	L3		3.3	quant.	91 (–)	97	85 (–)
5[e]	0.3			3.3	54	–	36	–
6	0.3			3.3	92[f]	92 (–)	65[g]	85 (–)
7	0.15			6.6	quant.	92 (–)	61	85 (–)
8	0.05			19.8	66	92 (–)	–	–
9	0.3			6.6	quant.	87 (–)	quant.	84 (–)
10[h]	0.3			6.6	quant. (86)[i]	85 (–)	96 (90)[i]	84 (–)
11	0.3			13.3	quant.	91 (–)	58	83 (–)
12	0.3			20	quant.	90 (–)	69	83 (–)
13	0.3			33	40	89 (–)	–	–
14	0.3		*p*-MeOPh (**1b**)	3.3	94	86	89	82
15	0.3		*p*-ClPh (**1c**)	3.3	75	90	72	85
16	0.3		*o*-BrPh (**1d**)	3.3	quant.	99 (–)	70	94
17[j]	0.3			6.6	87 (80)[i]	99 (–)	–	–
18[j,k]	0.3			6.6	79 (72)[i]	99 (–)	–	–
19	0.3			13.3	34	98 (–)	–	–
20	0.3		2-furanyl (**1e**)	3.3	96	86	quant.	87
21	0.3		Me (**1f**)	3.3	92	58	95	62

[a] Typical experiments were carried out with salmon testes DNA (1.3 mg mL^{-1}) in MOPS buffer (20 mM pH 6.5) for 3 days at 5 °C using 100 equiv of dimethyl malonate or 1000 equiv of nitromethane, with respect to enone. [b] Equivalents of substrate **1a–f** with respect to Cu^{II} ions. [c] Conversion values were determined by ^{1}H NMR spectroscopy and are the average of duplicate experiments (standard deviation: ±3%). [d] The *ee* values were determined by analytical chiral HPLC. [e] Reaction performed without DNA. [f] 40 equiv of dimethyl malonate. [g] 200 equiv of nitromethane. [h] Reaction performed on a 80-mg scale [i] Yield of isolated product after column chromatography. [j] Reaction performed on a 1-mmol scale. [k] Using recycled catalyst solution from entry 17. [l] Not determined.

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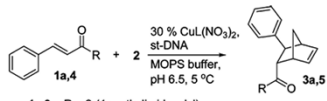
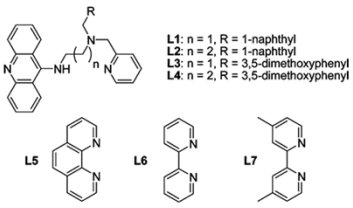
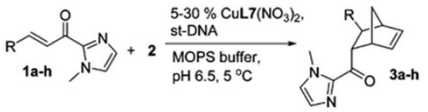




entry	ligand	% conversion[b] of **1a**	% ee[c] of **3a**	% ee[c–e] of **5**
1		<5	n.d.[f]	10 (+)
2	L1	61	10 (+)	48 (+)
3	L2	83	16 (−)	49 (−)
4	L3	78	29 (+)	37 (+)
5	L4	93	68 (+)	37 (+)
6	L5	17	68 (+)	72 (+)
7	L6	25	87 (+)	90 (+)
8	L7	90	97 (+)	99 (+)

[a] All experiments were carried out with salmon testes DNA (1.3 mg mL^{-1}, 2 mM in base pairs), 0.3 mM [Cu(L)$(NO_3)_2$] (30 mol %), 1 mM **1a** in 15 mL of MOPS buffer (20 mM, pH 6.5) for 3 days at 5 °C, unless noted otherwise. [b] Determined by ^{1}H NMR. [c] For the endo isomer. [d] All conversions >80%. [e] Data taken from ref 5. [f] n.d. = not determined.



entry	R (substrate)	endo:exo	% ee[b]
1	Ph (**1a**)	99:1	97 (+) (2*S*,3*S*)
2[c]	Ph (**1a**)	99:1	98 (+) (2*S*,3*S*)
3	*p*-$MeOC_6H_4$ (**1b**)	99:1	98
4[d]	*p*-ClC_6H_4 (**1c**)	n.d.	96
5[e]	*o*-BrC_6H_4 (**1d**)	96:4	94
6	2-furanyl (**1e**)	97:4	94
7	cyclohexyl (**1f**)	94:6	88
8	Me (**1g**)	>99:1	86
9[e]	Me (**1g**)	>99:1	88
10	H (**1h**)	98:2	80
11[e]	H (**1h**)	99:1	83

[a] All experiments were carried out with st-DNA (1.3 mg mL^{-1}, 2 mM in base pairs), 0.3 mM [Cu(**L7**)$(NO_3)_2$] (30 mol %), 2 mM **1** in 45 mL of MOPS buffer (20 mM, pH 6.5) for 3 days at 5 °C, unless noted otherwise. [b] For the endo isomer. [c] 1 mM **1**, 0.05 mM Cu(**L7**)$(NO_3)_2$ (5 mol %), 1.3 mg mL^{-1} st-DNA (2 mM in base pairs). [d] Conversion ~50%. [e] Conversion ~70%.

Org. Lett. **2007**, *9*, 3647–3650

Scheme 1. Cu−L$(NO_3)_2$/DNA Catalyzed Diels−Alder Cycloaddition between **1** and **2**

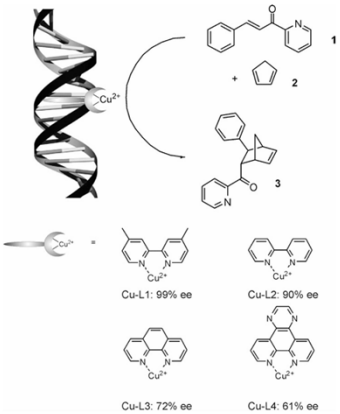


Table 1. k_{app} of the Diels-Alder Reaction of **1** with **2** Catalyzed by Copper Complexes Cu−L(1−3) with and without st-DNA[a]

		k_{app} ($M^{-1}s^{-1}$)		
entry	complex	without st-DNA	with st-DNA[b]	rate acceleration ($k_{app,with\ DNA}/k_{app,w/o\ DNA}$)
1	Cu−L1	0.0069 ± 0.0003	0.40 ± 0.04	58
2	Cu−L2	0.0092 ± 0.0004	0.022 ± 0.001	2.4
3	Cu−L3	0.010 ± 0.001	0.023 ± 0.001	2.3
4	$Cu(NO_3)_2$	0.095 ± 0.006	0.040 ± 0.004	0.4

[a] [Cu−L] = 0.15 mM, 25.0 °C, MOPS buffer (20 mM, pH = 6.5), [**1**] = 6.0×10^{-3} mM, [**2**] = 0.5 − 2.0 mM. [b] Ratio of Cu−L to base pairs DNA 1:6.

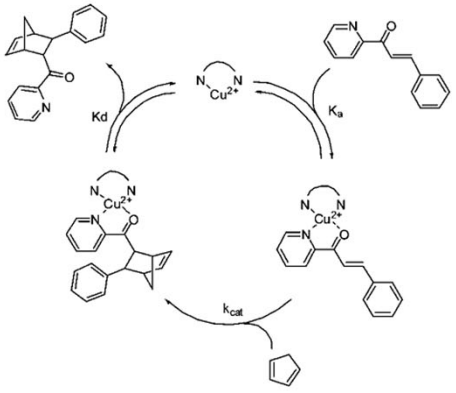


Table 2. Kinetic and Thermodynamic Parameters: K_a, k_{cat}, $k_{(-)}$, $k_{(+)}$, and the Isobaric Activation Parameters at 298 K[a,b]

	Cu−L1	Cu−L1/st-DNA[c]
K_a (M^{-1})	$(4.0 \pm 0.8) \times 10^2$	$(5.0 \pm 1.4) \times 10^2$
k_{cat} ($M^{-1}s^{-1}$)	$(4.5 \pm 1.2) \times 10^{-2}$	3.8 ± 0.8
$k_{(-)}$ ($M^{-1}s^{-1}$)	$(2.2 \pm 0.6) \times 10^{-2}$	$(5.8 \pm 1.2) \times 10^{-2}$
$k_{(+)}$ ($M^{-1}s^{-1}$)	$(2.2 \pm 0.6) \times 10^{-2}$	3.8 ± 0.8
$\Delta G^{\ddagger}$ (kcal/mol)	21 ± 1	18 ± 1
$\Delta H^{\ddagger}$ (kcal/mol)	10 ± 1	3.1 ± 0.5
$T\Delta S^{\ddagger}$ (kcal/mol)	-11 ± 1	-15 ± 1

[a] [Cu−L] = 0.10 − 0.25 mM, 25.0 °C, MOPS buffer (20 mM, pH = 6.5). [**1**] = 6.0 × 10^{-3} mM, [**2**] = 0.5 − 2.0 mM. [b] Conditions determination isobaric activation parameters; [Cu−L] = 0.1 mM, 25.0 °C, MOPS buffer (20 mM, pH = 6.5). [**1**] = 6.0 × 10^{-3} mM, [**2**] = 0.5 − 2.0 mM. See Supporting Information for the corresponding Eyring plots. [c] Fixed ratio of Cu-L1 to base pairs DNA 1:6.

entry	oligonucleotide	T_m (ΔT_m with Cu-L1) (°C)	K_a[a] (DNA)/M^{-1}	ee (%)[b,c]	k_{app}[d] ($M^{-1}s^{-1}$)
1	no DNA			<5	$(5.05 \pm 0.07) \times 10^{-3}$
		double stranded DNA:			
2	st-DNA	63.0 (−0.5)	7.26×10^3	98.5 (+)	$(2.37 \pm 0.05) \times 10^{-1}$
3	poly d(AT):poly d(AT)		n.d.[e]	15 (−)[g]	
4	poly d(GC):poly d(GC)		n.d.[f]	78 (+)[h]	
5	$d(GCGCGCGC)_2$	38.0 (−1.0)	9.42×10^3	86 (+)	$(2.98 \pm 0.38) \times 10^{-2}$
6	$d(GCGCGCGCGCGC)_2$	45.5 (−6.0)	8.37×10^3	95 (+)	$(6.45 \pm 0.84) \times 10^{-2}$
7	$d(GACTGACTAGTCAGTC)_2$	33.0 (−2.0)	9.55×10^3	78 (+)	$(4.44 \pm 0.32) \times 10^{-2}$
8	$d(CAGTCAGTACTGACTG)_2$	32.5 (−0.5)	1.26×10^4	83 (+)	$(3.39 \pm 0.05) \times 10^{-2}$
9	$d(TCGCGATCGCGA)_2$	35.0 (−6.0)	1.17×10^4	78 (+)	$(2.67 \pm 0.11) \times 10^{-2}$
10	$d(TCGCGTACGCGA)_2$	34.0 (−5.5)	1.31×10^4	82 (+)	$(1.49 \pm 0.05) \times 10^{-2}$
11	$d(TCGGAATTCCGA)_2$	23.0 (−1.5)	1.33×10^4	90 (+)	$(5.75 \pm 0.52) \times 10^{-2}$
12	$d(TCGGTTAACCGA)_2$	22.0 (−2.5)	1.15×10^4	95 (+)	$(7.03 \pm 0.97) \times 10^{-2}$
13	$d(TCGGGATCCCGA)_2$	28.0 (−4.5)	1.35×10^4	98.4 (+)	$(2.92 \pm 0.13) \times 10^{-1}$
14	$d(TCGGGTACCCGA)_2$	27.0 (−4.5)	1.32×10^4	98.6 (+)	$(2.94 \pm 0.44) \times 10^{-1}$
15	$d(TCAGGGCCCTGA)_2$	30.0 (−5.0)	9.59×10^3	99.4 (+)	$(5.00 \pm 0.27) \times 10^{-1}$
16	$d(AGGGCCCT)_2$	28.0 (−4.0)	5.90×10^3	97 (+)	$(1.35 \pm 0.05) \times 10^{-1}$
		Single stranded DNA:			
17	dNTP			18 (+)	$(2.50 \pm 0.10) \times 10^{-3}$
18	d(GGG)			<5 (+)	
19	d(CCC)			<5 (+)	
20	d(TCAGGGCACT)			81 (+)	$(4.87 \pm 0.09) \times 10^{-3}$

[a] Determined at 18 °C, error <5% in all cases, [b] The 0.3 mM Cu-L1, 2 mM base pairs DNA, in MOPS buffer (20 mM, pH 6.5), at 5 °C. One mM **1**, and 8 mM **2**. The ee values are the average of two experiments, >80% conversion to **3** after 60 h unless noted otherwise, [c] Endo:exo >98:2. [d] Performed under standard kinetic conditions. [Cu−L1] = 0.10 mM, [**1**] = 6.0×10^{-3} mM, [**2**] = 1.5 mM, temperature 18.0 °C. All measurements were performed 3×. [e] Not determined because a new complex was formed according to UV−vis spectroscopy. [f] Not determined because an irregular change in absorption was observed upon titration of oligomer. [g] Conversion 7%. [h] Conversion 15%.

J. Am. Chem. Soc. **2008**, *130*, 11783–11790

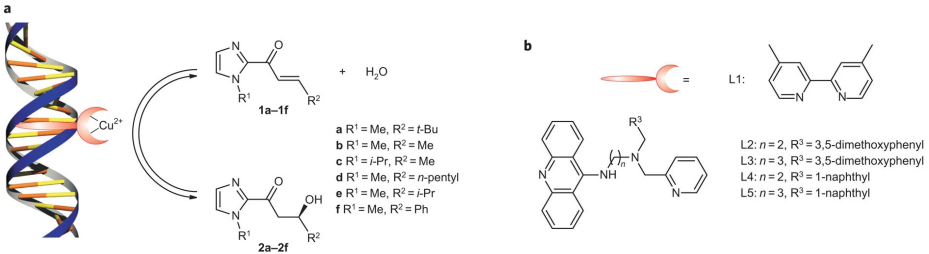
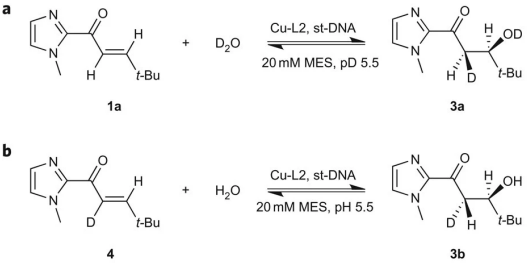
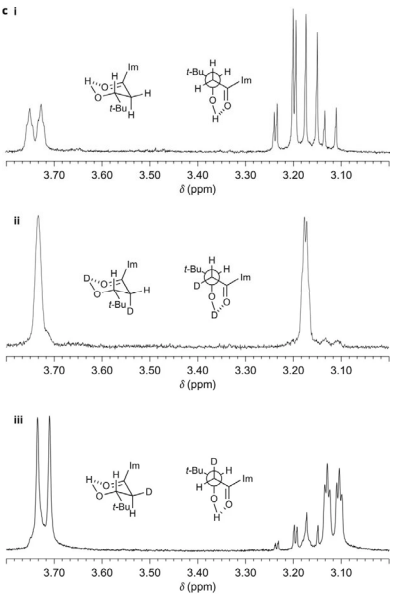


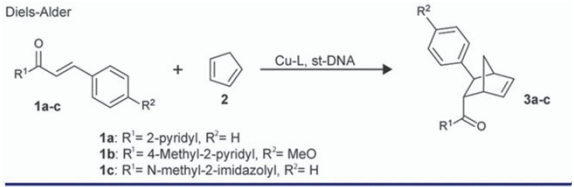
Table 1 | Reaction optimization and substrate scope.

Entry	Starting material	Product	Ligand	Reaction time (h)	Conversion* (%)	e.e.† (%)
1	**1a**	**2a**	**L1**	24	14	19 (*R*)
2	**1a**	**2a**	**L2**	24	55	72 (*R*)
3	**1a**	**2a**	**L3**	24	20	24 (*R*)
4	**1a**	**2a**	**L4**	24	36	55 (*R*)
5	**1a**	**2a**	**L5**	24	24	20 (*R*)
6‡	**1a**	**2a**	**L2**	72	33	62 (*R*)
7	**1a**	**2a**	-	24	20	42 (*S*)
8	**1b**	**2b**	**L2**	7	100	28
9	**1c**	**2c**	**L2**	7	100	3
10	**1d**	**2d**	**L2**	7	75	47
11	**1e**	**2e**	**L2**	7	71	60
12	**1f**	**2f**	**L2**	24	0	n.d.

Standard conditions: 5 °C, 20 mM MES buffer, pH 5.5, 15 μmol **1** (1 mM), 1.3 mg ml^{-1} st-DNA (2 mM base pairs), 0.39 mM ligand, 0.3 mM $Cu(NO_3)_2$, unless noted otherwise. *Determined by ^{1}H NMR spectroscopy. †Determined by HPLC using a chiral stationary phase. ‡0.14 mg ml^{-1} st-DNA, 0.039 mM ligand, 0.03 mM $Cu(NO_3)_2$. n.d. = not determined.


a
Cu-L2, st-DNA
20 mM MES, pD 5.5
D2O
1a
3a
b
Cu-L2, st-DNA
20 mM MES, pH 5.5
H2O
4
3b

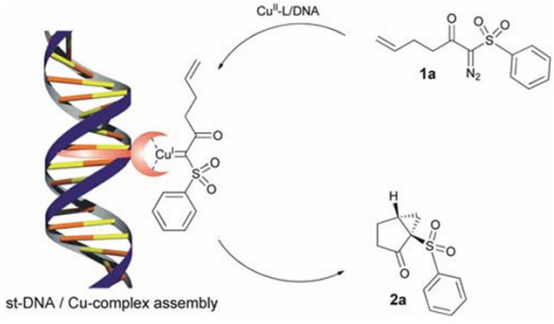

c i
ii
iii
3.70
3.60
3.50
3.40
3.30
3.20
3.10
δ (ppm)

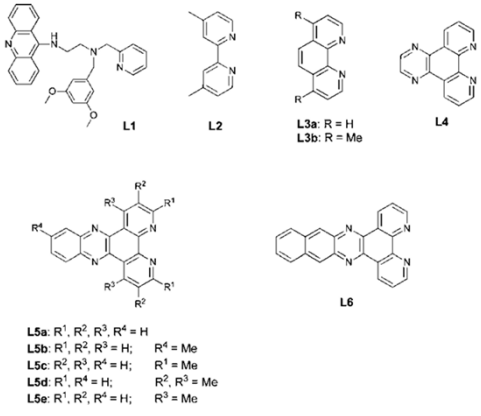


	Cu–L	Reactants		Conversion[b] (%)	*Endo* : *exo*[c]	ee[c] (%)
Diels–Alder reaction						
1[d]	Cu–L1	**1a**	**2**	Full	> 99 : 1	99 (+)
2[d]	Cu–L2	**1a**	**2**	Full	98 : 2	90 (+)
3	Cu–L3	**1a**	**2**	Full	98 : 2	89 (+)
4	Cu–L4	**1a**	**2**	Full	95 : 5	40 (−)
5	Cu–L5	**1a**	**2**	Full	99 : 1	95 (+)
6	Cu–L6	**1a**	**2**	Full	98 : 2	92 (+)
7	Cu–L7	**1a**	**2**	23	89 : 11	60 (−)
8	Cu–L8	**1a**	**2**	20	92 : 8	79 (−)
9	Cu–L9	**1a**	**2**	Full	94 : 6	28 (−)
10	Cu–L10	**1a**	**2**	17	92 : 8	71 (−)
11	Cu–L1	**1b**	**2**	25	> 99 : 1	> +99[e]
12	Cu–L7	**1b**	**2**	10	92 : 8	−92[e]
13[f]	Cu–L1	**1c**	**2**	> 80	99 : 1	97 (2*S*,3*S*)
14	Cu–L7	**1c**	**2**	15	82 : 18	42 (2*R*,3*R*)
Friedel–Crafts alkylation						
15[g]	Cu–L1	**1d**	**4**	Full	—	83 (+)
16	Cu–L7	**1d**	**4**	20	—	50 (−)

[a] Reactions performed with 1 mM substrate, 8 mM **2** (or 5 mM **4**), 0.3 mM Cu–L, 2 mg ml^{-1} st-DNA, in 20 mM mops buffer pH 6.5, for 3 days at 5 °C. All data averaged over two experiments. [b] Determined by ^{1}H-NMR. [c] Determined by HPLC. [d] Data from ref. 6*a*. [e] + and − sign correspond to elution order on the HPLC (first and second, respectively). [f] Data taken from ref. 6*b*. [g] Data taken from ref. 8.

Chem.Commun. **2012**, *48*, 2394–2396

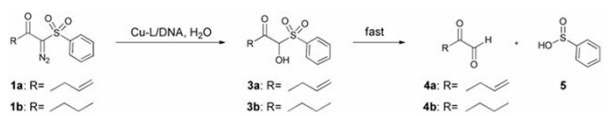




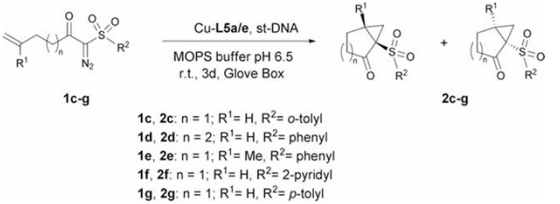
Chem. Sci. **2013**, *4*, 2013–2017

Entry	$Cu(NO_3)_2$ (mol%)	Ligand	Ligand (mol%)	Conversion[b] [%]	Yield[b] [%]	ee[b] [%]
Ligand to copper ratio 1 : 1[c]						
1[e]	15	—	—	45	17	0
2[f]	15	—	—	Full	76	0
3	15	—	—	38	0	0
4[g]	15	**L1**	15	42	0	0
5	15	**L2**	15	45	0	0
6	15	**L3a**	15	48	5	10
7	15	**L4**	15	63	10	28
8	15	**L5a**	15	73	19	37
9[h]	15	**L5a**	15	82	11	29
10	30	**L5a**	30	64	20	59
11[f]	15	**L5a**	15	Full	76	0
12	15	**L5b**	15	35	17	26
13	15	**L5c**	15	56	<5	<5
14	15	**L5d**	15	83	17	12
15	15	**L5e**	15	60	13	60
16	30	**L5e**	30	81	26	73
17[g]	15	**L6**	15	41	<5	<5
Ligand to copper ratio 2 : 1[d]						
18[i]	30	**L5a**/**L2**	30/30	80	20	61
19[i]	30	**L5a**/**L3b**	30/30	Full	28	76
20[g]	30	**L5a**	60	62	19	67
21[g]	30	**L5e**	60	62	30	84
22[g,j]	30	**L5e**	60	Full	46	83

[a] The experiments were carried out in a glove box, with 1 mM **1a**, 1.5 mM base pairs of st-DNA and the indicated concentration of Cu-complex in deoxygenated 10 mM MOPS buffer (pH 6.5), 2% v/v DMF, for 3 days at room temperature, unless otherwise specified. [b] Conversions, yields and enantioselectivities are based on areas of HPLC peaks that are compared to methyl phenyl sulfone as an external standard. All data were averaged over two experiments. Product **2a** was obtained in (1*R*,5*R*)-configuration by comparison of the elution order with those reported previously.[38] [c] Reproducibility: ee values and yields ±5%, conversions ±10%. [d] Reproducibility: ee values ±3%, yields ±5%, conversions ±10%. [e] Non-deoxygenated solution without DNA. [f] Without DNA. [g] Complex pre-formed in DMF prior to the reaction. [h] With 2 mM sodium ascorbate. [i] 30 mol% (0.30 mM) of isolated Cu-**L5a**, 0.30 mM of ligand **L2** or **L3b** added after incubation of Cu-**L5a** complex with st-DNA for 3 h. [j] 6 days reaction time.

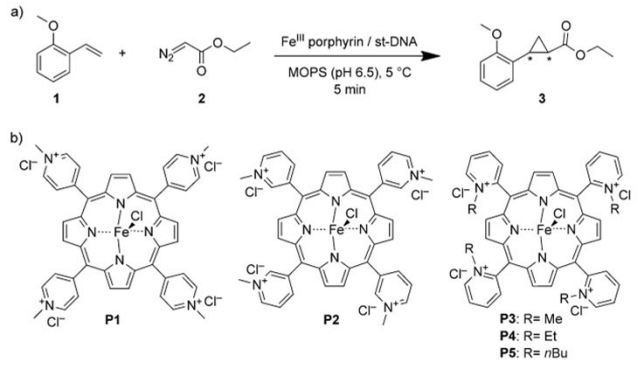


Scheme 2 O–H bond insertion with Cu-L/DNA in water and subsequent decomposition. For **1b**: catalytic reaction with 1 mM **1b** and 0.15 mM Cu-**L5a** in water.



Entry	Substrate	Conversion[b] [%]	Yield[b] [%]	ee[b] [%]
1	**1c**	78	0	n.d.
2	**1d**	26	0	n.d.
3[c]	**1e**	n.d.	2	n.d.
4[c,d]	**1e**	n.d.	40	16
5[c]	**1f**	n.d.	13	51
6[c]	**1g**	n.d.	29	63 (1*R*,5*R*)

[a] The experiments were carried out in a glove box, with 1 mM **1a**, 1.5 mM base pairs of st-DNA, 30 mol% (0.30 mM) of $Cu(NO_3)_2$ and 0.30 mM of **L5a** mixed in DMF prior to the reaction, in 10 mM of deoxygenated MOPS buffer (pH 6.5), 2% v/v DMF, for 3 days at room temperature, unless otherwise specified. n.d. = not determined. [b] Conversions, yields and enantioselectivities are based on areas of HPLC peaks that are compared to methyl phenyl sulfone as external standard. All data are averaged over two experiments. Reproducibility: ee values and yields ±5%, conversions ±10%. [c] 30 mol% (0.30 mM) of $Cu(NO_3)_2$ and 0.60 mM of **L5e** mixed in DMF prior to the reaction. [d] With 2 mM sodium ascorbate.

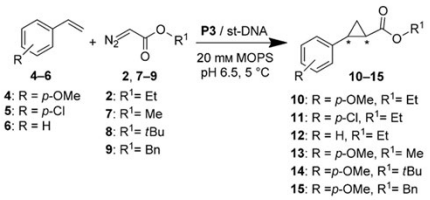


Scheme 1. a) Enantioselective cyclopropanation reaction catalyzed by a DNA-based iron porphyrin. b) Iron(III) complexes of *meso*-tetrakis(*N*-alkylpyridyl)porphyrins used in this study.

Table 1: Enantioselective cyclopropanation of **1** with EDA under the catalysis of iron porphyrins in the presence and absence of st-DNA.[a]

Entry	Porphyrin	st-DNA [mM]	Yield [%][b]	TTN[c]	*ee* [%][b]
1	**P1**	–	≤3	–	–
2	**P1**	6	≤3	–	–
3	**P2**	–	≤3	–	–
4	**P2**	6	≤3	–	–
5	**P3**	–	≤3	–	–
6	**P3**	6	14±2	9	42
7[d]	**P3**	6	16±4	22	40
8[e]	**P3**	–	≤3	–	–
9	**P3**	3	22±1	14	33
10	**P3**	1.5	25±0	17	24
11	**P4**	–	33±3	22	–
12	**P4**	6	15±0.5	10	23
13	**P5**	–	26±0	17	–
14	**P5**	6	11±1	7	5

[a] The experiments were carried out with **1** (5 mM), **2** (50 mM), st-DNA (6 mM in base pairs), and the Fe^{III} porphyrin (75 μM) in 20 mM MOPS buffer (pH 6.5) containing acetonitrile (3% v/v) for 5 min at 5 °C, unless otherwise specified. Yields and *ee* values are based on the areas of HPLC and GC peaks as compared to those of 2-methyl anisole as an internal standard. All data were averaged over two or more experiments. Errors reported are standard deviations. [b] The yield and *ee* value (reproducibility: ±3% *ee*) of the *trans* isomer are given. The diastereomeric ratio of the product ranged from 88:12 to 95:5. [c] TTN = total turnover number. [d] Metalloporphyrin concentration: 37.5 μM. [e] Metalloporphyrin concentration: 7 μM.



Scheme 2. Investigation of the scope of the enantioselective cyclo-

Table S2. Investigation of the scope of diazocompounds.[a]

Entry	Styrene	Diazo reagent	Product	DNA [mM]	Yield [%][b]	TTN[c]	ee_{trans} [%][b]
1	**4**	**7**	**13**	-	24±9	31	-
2	**4**	**7**	**13**	6	44±3	59	50
3	**4**	**8**	**14**	-	≤3	-	-
4	**4**	**8**	**14**	6	12±5	15	21
5	**4**	**9**	**15**	-	≤3	-	-
6	**4**	**9**	**15**	6	≤3	-	-

[a] The experiments were carried out with 5 mM substrate and 50 mM diazo compound, 6 mM base pairs of st-DNA and 37.5 μM of P3 in 20 mM MOPS buffer (pH 6.5), 3% v/v ACN, for 5 minutes at 5°C, unless otherwise specified. Yields and enantioselectivities are based on areas of HPLC and GC peaks that are compared to 2-methyl anisole as internal standard. All data were averaged over two or more experiments. [b] of the trans isomers; reproducibility *ee*'s ±3%. [c] TTN = total turnover numbers.

Table 2: Investigation of the scope of st-DNA/**P3**-catalyzed enantioselective cyclopropanation reactions with **2**.[a]

Entry	Styrene	Product	DNA [mM]	Yield [%][b]	TTN	*ee* [%][b]
1	**4**	**10**	–	3 ± 3	4	–
2	**4**	**10**	6	32 ± 6	43	50
3	**5**	**11**	–	≤ 3	–	–
4	**5**	**11**	6	4 ± 1	5	41
5	**6**	**12**	–	≤ 3	–	–
6	**6**	**12**	6	12	16	53

[a] The experiments were carried out with the styrene substrate (5 mM), **2** (50 mM), st-DNA (6 mM in base pairs), and **P3** (37.5 μM) in 20 mM MOPS buffer (pH 6.5) containing acetonitrile (3% v/v) for 5 min at 5 °C. Yields and *ee* values were determined by HPLC and GC with 2-methyl anisole as an internal standard. All data were averaged over two or more experiments. Errors reported are standard deviations. [b] The yield and *ee* value (reproducibility: ± 3% *ee*) of the *trans* isomer are given.

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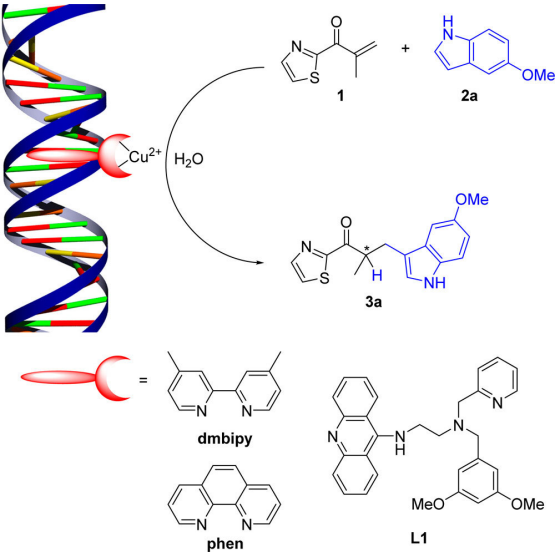

Table 1. Results of the Catalytic Friedel–Crafts Conjugate Addition/Enantioselective Protonation Reaction of 1 with 2a[a]

entry	catalyst	[st-DNA] (mM)	conv[b] (%)	ee[b] (%)
1	$[Cu(NO_3)_2].3H2O$	1	41	<5
2	$[Cu(NO_3)_2].3H2O$		14	
3	$[Cu(dmbipy)(NO_3)_2]$	1	90	59
4	$[Cu(dmbipy)(NO_3)_2]$		1	
5	$[Cu(phen)(NO_3)_2]$	1	71	<5
6	$[Cu(L1)(NO_3)_2]$	1	66	−53

[a]Typical reaction conditions: **1** (1 mM), **2a** (1 mM), Cu-dmbipy (0.15 mM), st-DNA (1 mM in base pairs) in 20 mM MES pH 5.0 at 4 °C, for 18 h. [b]Conversion (reproducibility ±5%) and ee values (reproducibility ±3%) were determined by HPLC.

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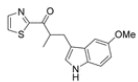
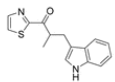
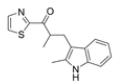
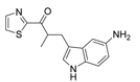
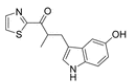
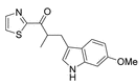
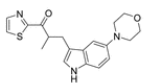
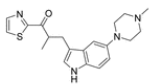
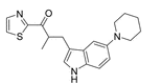
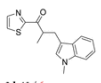
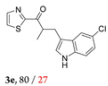
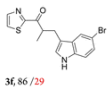
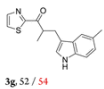
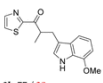
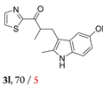
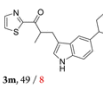
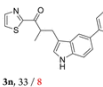
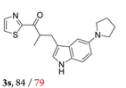
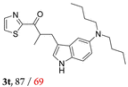
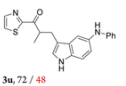
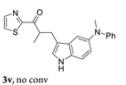
Entry	Product, yield[b] / ee (%)[c]
1	 **3a**, 81 / 59
2	 **3b**, 54 / 32
3	 **3c**, 65 / <5
8	 **3h**, 43 / 52
9	 **3i**, 66 / 41
10	 **3j**, 75 / 11
15	 **3p**, 83 / 84
16	 **3q**, 40 / 74
17	 **3r**, 87 / 79
4	 **3d**, 45 / 6
5	 **3e**, 80 / 27
6	 **3f**, 86 /29
7	 **3g**, 52 / 54
11	 **3k**, 77 / 29
12	 **3l**, 70 / 5
13	 **3m**, 49 / 8
14	 **3n**, 33 / 8
18	 **3s**, 84 / 79
19	 **3t**, 87 / 69
20	 **3u**, 72 / 48
21	 **3v**, no conv

[a]Typical reaction conditions: **1** (1 mM), **2a–v** (1 mM), Cu-dmbipy (0.15 mM), st-DNA (1 mM in base pairs) in 20 mM MES pH 5.0 at 4 °C, for 18 h. [b]Isolated yields after column chromatography. [c]Ee values determined by HPLC (reproducibility ±3%).

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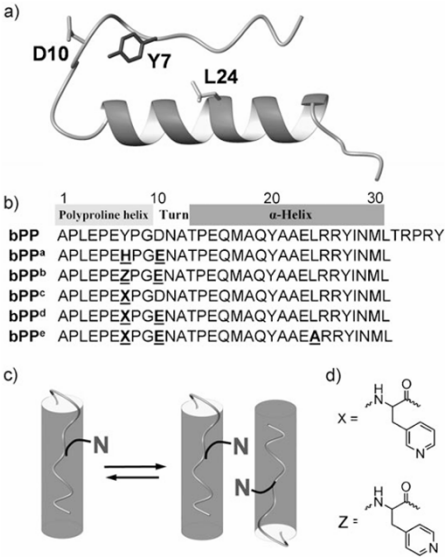

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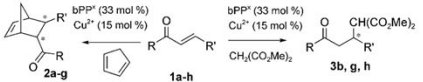


a: R = 2-pyridyl, R' = Ph
b: R = 2-*N*-methylimidazolyl, R' = Ph
c: R = 2-*N*-methylimidazolyl, R' = *p*-$MeOC_6H_4$
d: R = 2-*N*-methylimidazolyl, R' = *p*-ClC_6H_4
e: R = 2-*N*-methylimidazolyl, R' = *o*-BrC_6H_4
f: R = 2-*N*-methylimidazolyl, R' = 2-furanyl
g: R = 2-*N*-methylimidazolyl, R' = Me
h: R = 2-*N*-methylimidazolyl, R' = *i*Pr

Table 1: Results of Diels–Alder reactions catalyzed by Cu–**bPPˣ** (Scheme 1).[a]

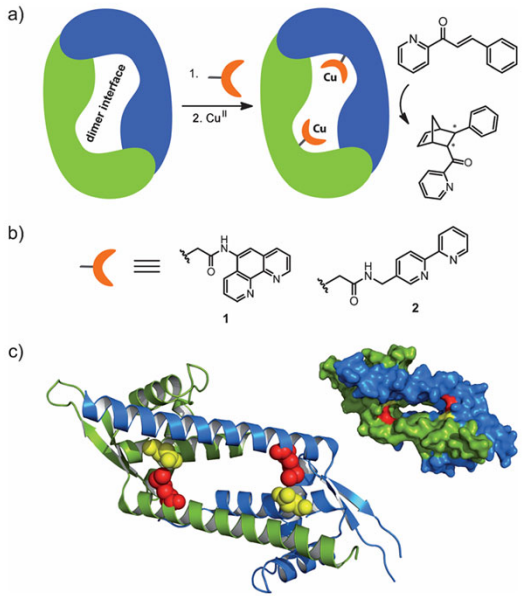
Entry	Substrate	**bPP**[a] *ee* [%][b] (conv. [%])	**bPP**[b] *ee* [%][b] (conv. [%])	**bPP**[c] *ee* [%][b] (conv. [%])	**bPP**[d] *ee* [%][b] (conv. [%])	**bPP**[e] *ee* [%][b] (conv. [%])
1	**1a**	<3 (96)	6 (65)[d]	83 (73)	80 (full)	4 (94)
2[c]	**1a**				74 (full)	
3	**1b**		<3 (32)	65 (full)	14 (58)	<3 (full)
4	**1c**			62 (3)	52 (9)	3 (7)
5	**1d**			39 (8)	23 (10)	4 (4)
6	**1e**			20 (3)	8 (<3)	3 (21)
7	**1f**			13 (<3)	8 (<3)	9 (<3)
8	**1g**			<3 (full)	<3 (96)	

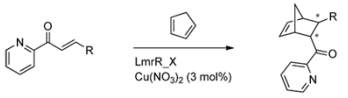
[a] Typical conditions: 200 μM **bPPˣ** and 95 μM $Cu(H_2O)_6(NO_3)_2$ in 20 mM MOPS buffer (pH 6.5) for 3 days at 5 °C, unless noted otherwise. The *ee* values are the average from 2 experiments and are reproducible within 2%. In all cases, the (−) enantiomer was found in excess. [b] For the *endo* isomer, *endo*/*exo* ≥ 95:5. [c] 5 mol% Cu^{2+} and 11 mol% **bPP**[d].

Table 2: Results of Michael addition reactions catalyzed by Cu–**bPPˣ** (Scheme 1).[a]

Entry	Substrate	**bPP**[c] *ee* [%][b] (conv. [%])	**bPP**[d] *ee* [%][b] (conv. [%])	**bPP**[e] *ee* [%][b] (conv. [%])
1	**1b**	<3 (25)	<3 (59)	<3 (15)
2	**1g**	66 (70)	86 (85)	65 (90)
3[c]	**1g**		75 (70)	
4	**1h**	<3 (10)	6 (54)	

[a] Typical conditions: 200 μM **bPPˣ** and 95 μM $Cu(H_2O)_6(NO_3)_2$ (15 mol%; **bPPˣ** to Cu^{II} ratio: 2.1) in 20 mM MOPS buffer (pH 6.5) for 3 days at 5 °C, unless noted otherwise. [b] The *ee* values are the average of 2 experiments and are reproducible within 2%. [c] 5 mol% Cu^{2+} and 11 mol% **bPP**[d].



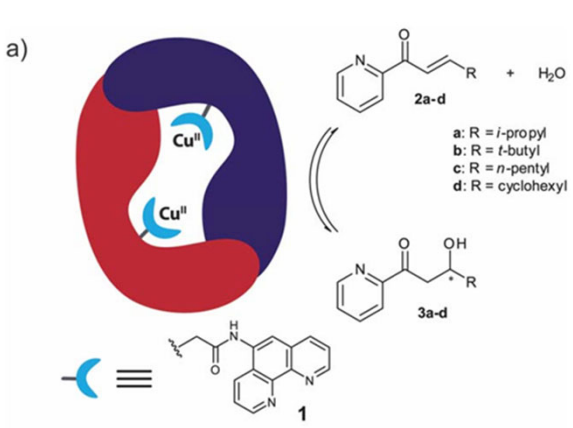


a: = Ph
b: = *m*-MeOPh
c: = Me

Scheme 1. Asymmetric Diels–Alder reaction catalyzed by LmrR_X/$Cu(NO_3)_2$ complexes in a MOPS buffered solution.

Entry	Catalyst	Product	Conversion [%]	*endo*:*exo*	*ee* (*endo*) [%]
1	LmrR_N19C_**1**_Cu^{II}	**4a**	24±3	92:8	53±5 (+)
2	LmrR_M89C_**1**_Cu^{II}	**4a**	93±4	95:5	97±1 (+)
3[b]	LmrR_M89C_**1**_Cu^{II}	**4a**	98±1	90:10	95±1 (+)
4	LmrR_M89C_**1**_Cu^{II}	**4b**	56±9	96:4	93±1 (+)
5	LmrR_M89C_**1**_Cu^{II}	**4c**	97±1	n.a.[c]	<5
6	LmrR_M89C_**2**_Cu^{II}	**4a**	55±8	63:37[d]	66±2 (−)
7	LmrR_M89C_V15A_**1**_Cu^{II}	**4a**	89±4	96:4	97±1 (+)
8	LmrR_M89C_D100E_**1**_Cu^{II}	**4a**	38±4	88:12	40±2 (+)
9	LmrR_M89C_D100E_**1**_Cu^{II}	**4b**	14±2	84:14	21±3 (+)
10	LmrR_M89C_Cu^{II}	**4a**	30±5	90:10	<5
11[e]	LmrR_9_Cu^{II}	**4a**	23±1	88:12	13±4 (+)
12[f]	LmrR_Phenanthroline_Cu^{II}	**4a**	29±8	90:10	28±7 (+)
13	Phenanthroline_Cu^{II}	**4a**	20±5	93:7	0
14	Phenanthroline_Cu^{II}	**4c**	full	95:5	0

[a] Typical conditions: 90% $Cu(H_2O)_6(NO_3)_2$ (3 mol%; 30 μM) loading with respect to LmrR_X in 20 mM MOPS buffer (pH 7.0), 150 mM NaCl, for 3 days at 4°C. Conversions and *ee* values are an average of two independent experiments, both carried out in duplicate. [b] Reaction carried out at room temperature. [c] *exo* peak could not be observed. [d] *ee exo* 90±2%. [e] Compound **9** was added 2:1 with respect to LmrR (wild-type). [f] Copper phenanthroline was added 2:1 with respect to LmrR (wild-type).


a)
Cu^{II}
Cu^{II}
2a-d
a: R = i-propyl
b: R = t-butyl
c: R = n-pentyl
d: R = cyclohexyl
3a-d
1

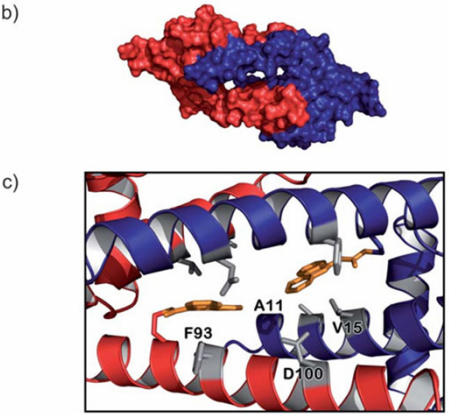
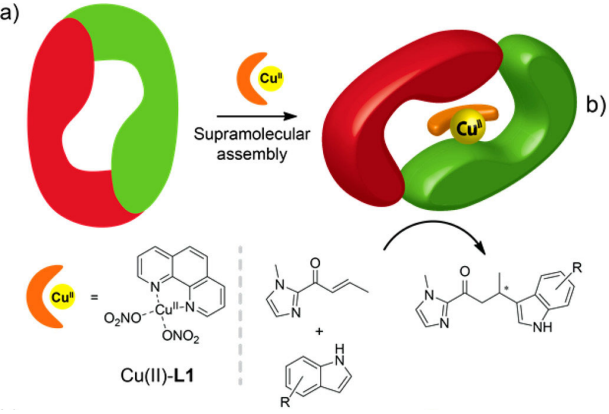
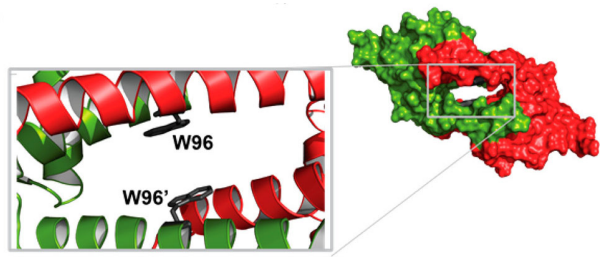

b)
c)
A11
F93
V15
D100

Table 1 Results of 1,4 addition of water to α,β-unsaturated ketones catalyzed by LmrR_LM_M89C_**1**_Cu^{II}[a]

Entry	Catalyst	Substrate	Product	Reaction time (hours)	Conversion (%)	ee (%)
1	LmrR_LM_M89C_**1**_Cu^{II}	**2a**	**3a**	16	29 ± 0	79 ± 0
2	LmrR_LM_M89C_**1**_Cu^{II}	**2a**	**3a**	40	67 ± 9	77 ± 2
3	LmrR_LM_M89C_**1**_Cu^{II}	**2a**	**3a**	64	82 ± 8	75 ± 2
4	LmrR_LM_M89C_**1**_Cu^{II}	**2a**	**3a**	112	82 ± 6	55 ± 3
5	LmrR_LM_M89C_**1**_Cu^{II}	**2b**	**3b**	16	17 ± 4	72 ± 9
6	LmrR_LM_M89C_**1**_Cu^{II}	**2b**	**3b**	64	80 ± 9	84 ± 2
7	LmrR_LM_M89C_**1**_Cu^{II}	**2c**	**3c**	16	57 ± 3	67 ± 3
8	LmrR_LM_M89C_**1**_Cu^{II}	**2c**	**3c**	40	90 ± 8	57 ± 5
9	LmrR_LM_M89C_**1**_Cu^{II}	**2d**	**3d**	40	40 ± 5	64 ± 5
10	LmrR_LM_M89C_**1**_Cu^{II}	**2e**	**3e**	40	<5	nd
11	LmrR_LM_M89C_A11V_**1**_Cu^{II}	**2a**	**3a**	40	73 ± 13	70 ± 4
12	LmrR_LM_M89C_V15A_**1**_Cu^{II}	**2a**	**3a**	40	50 ± 16	37 ± 1
13	LmrR_LM_M89C_F93A_**1**_Cu^{II}	**2a**	**3a**	40	45 ± 18	11 ± 4
14	LmrR_LM_M89C_F93Y_**1**_Cu^{II}	**2a**	**3a**	40	49 ± 4	71 ± 1
15	LmrR_LM_M89C_F93I_**1**_Cu^{II}	**2a**	**3a**	40	42 ± 8	71 ± 5
16	LmrR_LM_M89C_F93W_**1**_Cu^{II}	**2a**	**3a**	40	17 ± 3	41 ± 5
17	LmrR_LM_M89C_D100A_**1**_Cu^{II}	**2a**	**3a**	40	28 ± 1	<5
18	LmrR_LM_M89C_D100E_**1**_Cu^{II}	**2a**	**3a**	40	63 ± 9	62 ± 1
19	LmrR_LM_M89C_D100N_**1**_Cu^{II}	**2a**	**3a**	40	32 ± 10	<5
20	LmrR_LM_M89C_D100H_**1**_Cu^{II}	**2a**	**3a**	40	18 ± 8	<5
Control experiments[b]						
21	Cu^{II}-Phen[c]	**2a**	**3a**	40	81 ± 2	—
22	Cu^{II}-Phen[c]	**2b**	**3b**	16	67 ± 1	—
23	Cu^{II}-Phen[c]	**2b**	**3b**	40	88 ± 3	—
24	Cu^{II}-Phen[c]	**2c**	**3c**	40	27 ± 2	—
25	Cu^{II}-Phen[c]	**2d**	**3d**	40	37 ± 4	—
26	LmrR_LM_M89C_**1** (no Cu^{II})	**2a**	**3a**	40	8 ± 1	—
27	none	**2a**	**3a**	40	12 ± 3	—

[a] Typical conditions: 90% $Cu(H_2O)_6(NO_3)_2$ (3 mol%, 30 μM) loading with respect to LmrR_LM_X in 20 mM MOPS buffer (pH 7.0), 150 mM NaCl, at 4 °C. Conversions and ee values are determined by np-HPLC (conversions are corrected for the difference in molar absorption coefficient of starting material and product, see ESI†). Results are obtained as an average of two independent experiments, both carried out in duplicate. [b] Reactions performed with $Cu(H_2O)_6(NO_3)_2$ without LmrR generated significant amounts of side products. [c] $[Cu(Phen)(NO_3)_2]$.





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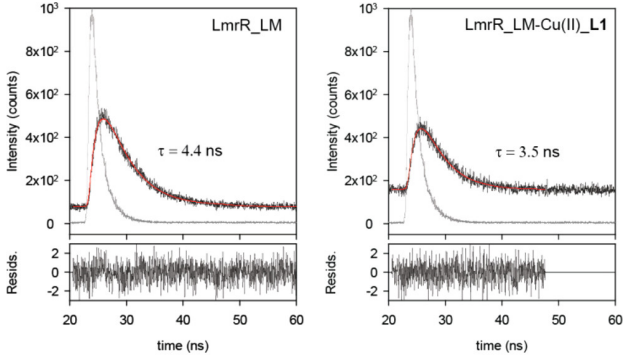
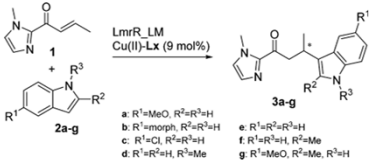


Protein	[Cu(II)-**L1**) (μM)	Lifetime (ns)
LmrR_LM	0	4.4
LmrR_LM	44	3.5
LmrR_LM_W96A	0	3.7
LmrR_LM_W96A	44	3.7

J. Am. Chem. Soc. **2015**, *137*, 9796−9799

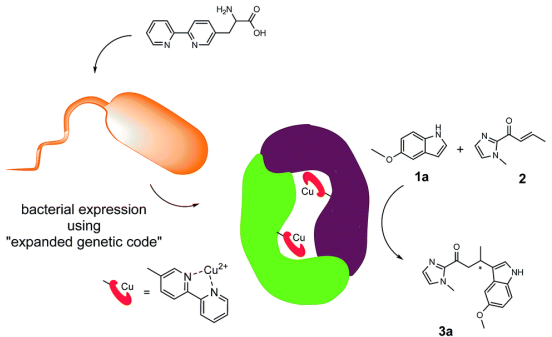
Table 1. Results of Friedel−Crafts Alkylation Reactions Catalyzed by LmrR-Based Artificial Metalloenzymes[a]



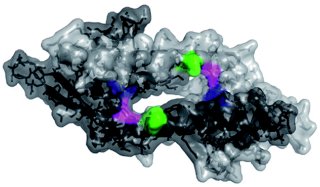
entry	indole	equiv of indole	product	conversion (%)[b]	ee (%)[c]
LmrR_LM ⊂ Cu(II)-**L1**					
1	**2a**	5	**3a**	57 ± 7	61 ± 8(+)
2	**2a**	1	**3a**	84 ± 5	75 ± 13(+)
3	**2a**	0.5	**3a**	90 ± 2[d]	84 ± 3(+)
4	**2b**	1	**3b**	94 ± 4	82 ± 1
5	**2c**	1	**3c**	19 ± 8	64 ± 7(+)
6	**2d**	1	**3d**	79 ± 2	18 ± 2(−)-R
7	**2e**	1	**3e**	full	94 ± 0(+)-R
8	**2f**	1	**3f**	full	93 ± 1
9	**2f**	0.5	**3f**	full[d]	93 ± 1
10	**2g**	1	**3g**	56 ± 25[e]	81 ± 1
LmrR_LM_W96A ⊂ Cu(II)-**L1**					
11	**2a**	1	**3a**	53 ± 28	<5

[a]Typical conditions: 75% Cu(II)-**L1** (9 mol %; 90 μM) loading with respect to LmrR_LM or LmrR_LM_W96A, 1 mM **1**, 0.5−5 mM **2**, in 20 mM MOPS buffer (pH 7.0), 150 mM NaCl, at 4 °C for 16 h (**2a**) or 64 h (other indoles). Conversions and ee values are an average of at least two independent experiments, both carried out in duplicate. [b]Conversions are determined by HPLC and are based on substrate **1**. [c]Sign of rotation and absolute configuration based on elution order in chiral HPLC.[9] [d]Conversion is based on substrate **2**.[11] [e]Additional unidentified products were detected (Figure S4).

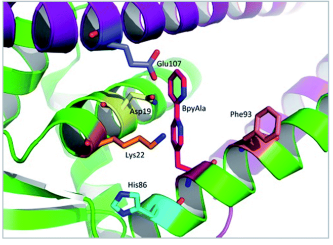
a)

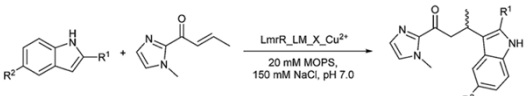

bacterial expression
using
"expanded genetic code"
Cu
Cu
1a
2
3a

b)



c)


Glu107
Asp19
BpyAla
Phe93
Lys22
His86



Scheme 1 Scope of the catalyzed Friedel–Crafts alkylation reactions.

Table 2 Scope of the vinylogous Friedel–Crafts alkylation reaction catalyzed by LmrR_LM_X_Cu^{II}[a]

Entry	Catalyst	Substrate	Product	Conversion (%)	ee (%)
1	LmrR_LM_M89X_Cu^{II}	**1b**	**3b**	16 ± 6	52 ± 3 (+)
2	LmrR_LM_M89X_H86A_Cu^{II}	**1b**	**3b**	11 ± 0	49 ± 3 (+)
3	LmrR_LM_M89X_F93W_Cu^{II}	**1b**	**3b**	16 ± 6	55 ± 3 (+)
4	LmrR_LM_M89X_Cu^{II}	**1c**	**3c**	2 ± 1	21 ± 1 (+)
5	LmrR_LM_M89X_H86A_Cu^{II}	**1c**	**3c**	5 ± 0	29 ± 0 (+)
6	LmrR_LM_M89X_F93W_Cu^{II}	**1c**	**3c**	3 ± 1	50 ± 2 (+)
7	LmrR_LM_M89X_Cu^{II}	**1d**	**3d**	92 ± 4	80 ± 2 (+)
8	LmrR_LM_M89X_H86A_Cu^{II}	**1d**	**3d**	79 ± 2	68 ± 2 (+)
9	LmrR_LM_M89X_F93W_Cu^{II}	**1d**	**3d**	94 ± 8	83 ± 0 (+)

[a] Typical conditions: 9 mol% $Cu(H_2O)_6(NO_3)_2$ (90 μM) loading with 1.25 eq. LmrR_LM_X (in monomer) in 20 mM MOPS buffer (pH 7.0), 150 mM NaCl, for 3 days at 4 °C. All data are the average of 2 independent experiments, each carried out in duplicate.

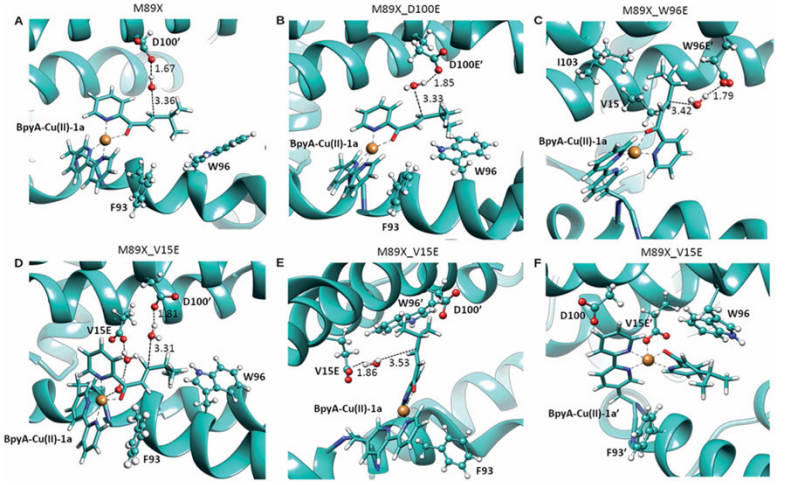
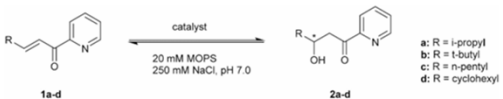


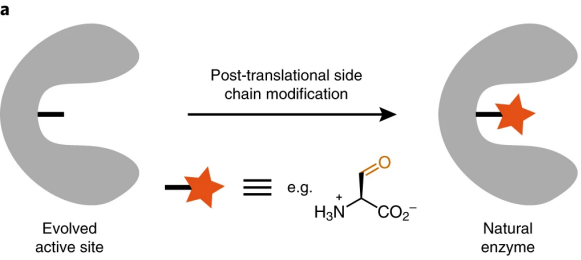
Fig. 3 Pre-reactive conformations from molecular dynamics simulations of (A) LmrR_M89X, (B) LmrR_M89X_D100E, (C) LmrR_M89X_W96E and (D–F) LmrR_M89X_V15E. Pre-reactive conformations are defined on the basis of the closeness of a water molecule to the β carbon of the substrate and the hydrogen-bonding to an aspartate or glutamate residue. Similar pre-reactive conformations are reached in 10% of the simulation by LmrR_M89X, 10% by LmrR_M89X_D100E, 91% LmrR_M89X_W96E and 31% by LmrR_M89X_V15E (Table S3†). The BpyA–Cu(II)–**1a** force field parameters were calculated with programs MCPB for the bonding terms and RESP for atomic charges, both from the AMBER program package.[58]

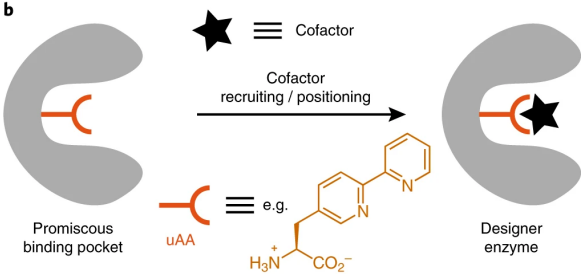
Table 1 Results of the enantioselective hydration reaction catalyzed by LmrR_X_Cu(II)[a]

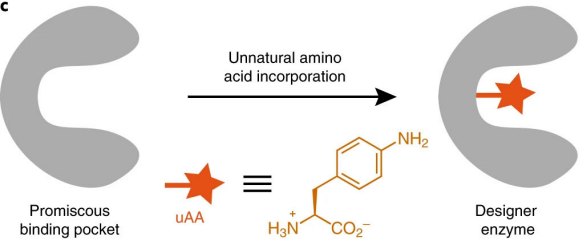


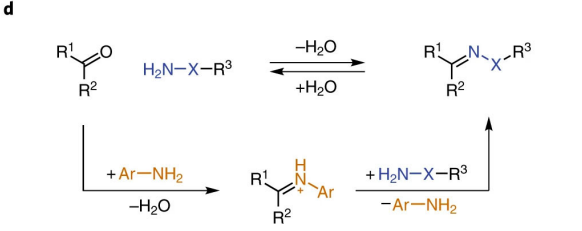
Entry	Catalyst	Substrate	Product	Conversion (%)	ee (%)
1	—	**1a**	**2a**	11 ± 3	—
2	$Cu(NO_3)_2$	**1a**	**2a**	83 ± 9	—
3	LmrR_M89X (no Cu(II))	**1a**	**2a**	9 ± 3	—
4	LmrR_M89X_Cu(II)	**1a**	**2a**	39 ± 7	42 ± 6
5	LmrR_M89X_D100E_Cu(II)	**1a**	**2a**	50 ± 6	30 ± 1
6	LmrR_M89X_D100Q_Cu(II)	**1a**	**2a**	35 ± 3	<5
7	LmrR_M89X_V15E_Cu(II)	**1a**	**2a**	75 ± 9	64 ± 2
8	LmrR_M89X_V15Q_Cu(II)	**1a**	**2a**	28 ± 5	15 ± 3
9	LmrR_M89X_W96E_Cu(II)	**1a**	**2a**	79 ± 3	6 ± 4
10	LmrR_M89X_W96Q_Cu(II)	**1a**	**2a**	64 ± 1	6 ± 1
Substrate scope[b]					
11	$Cu(NO_3)_2$	**1b**	**2b**	86 ± 4	—
12	LmrR_M89X_Cu(II)	**1b**	**2b**	37 ± 2	41 ± 4
13	LmrR_M89X_V15E _Cu(II)	**1b**	**2b**	64 ± 8	50 ± 2
14	$Cu(NO_3)_2$	**1c**	**2c**	26 ± 4	—
15	LmrR_M89X_Cu(II)	**1c**	**2c**	24 ± 4	19 ± 5
16	LmrR_M89X_V15E_Cu(II)	**1c**	**2c**	58 ± 5	14 ± 1
17	$Cu(NO_3)_2$	**1d**	**2d**	37 ± 12	—
18	LmrR_M89X_Cu(II)	**1d**	**2d**	17 ± 4	22 ± 2
19	LmrR_M89X_V15E_Cu(II)	**1d**	**2d**	45 ± 5	57 ± 3

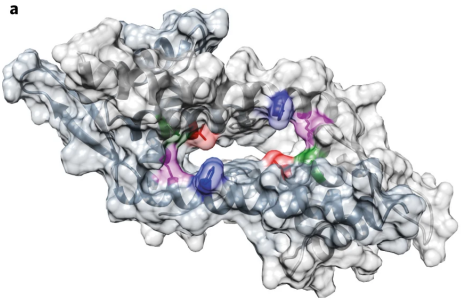
[a] Standard conditions: 9 mol% $Cu(H_2O)_6(NO_3)_2$ (90 μM) loading with 1.25 eq. LmrR_X (in monomer) in 20 mM MOPS buffer (pH 7.0), 250 mM NaCl, for 3 days at 4 °C. All data are the average of 2 independent experiments, each carried out in duplicate. Errors are reported as standard deviation.
[b] Conditions the same as in the experiments with **1a**.

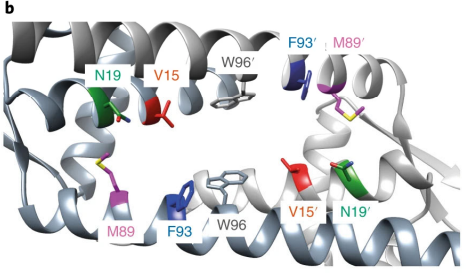

a
Post-translational side chain modification
e.g.
Evolved active site
Natural enzyme

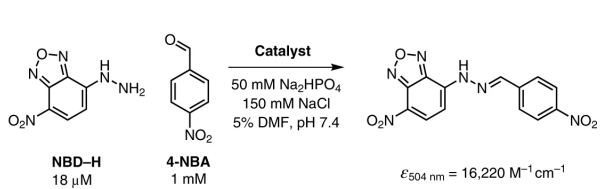

b
Cofactor
Cofactor recruiting / positioning
e.g.
uAA
Promiscous binding pocket
Designer enzyme

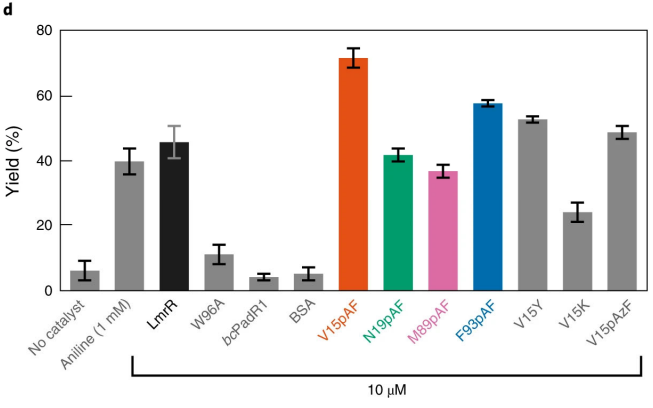

c
Unnatural amino acid incorporation
uAA
Promiscous binding pocket
Designer enzyme


d
–H2O
+H2O
+ Ar—NH2
–H2O
+ H2N—X—R3
– Ar—NH2


a


b
N19
V15
W96′
F93′
M89′
M89
F93
W96
V15′
N19′


c
Catalyst
50 mM Na2HPO4
150 mM NaCl
5% DMF, pH 7.4
NBD–H
18 μM
4-NBA
1 mM
ε504 nm = 16,220 M−1 cm−1


d
Yield (%)
80
60
40
20
0
No catalyst
Aniline (1 mM)
LmrR
W96A
bcPadR1
BSA
V15pAF
N19pAF
M89pAF
F93pAF
V15Y
V15K
V15pAzF
10 μM

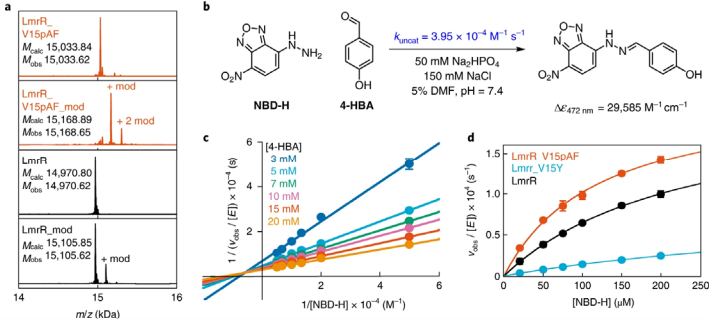


Table 1 | Catalytic parameters obtained from saturation kinetic studies for LmrR, LmrR_V15Y and LmrR_V15pAF

	Hydrazone formation*				NBD-ONH_2 hydrolysis				Oxime formation†			
	k_{cat}	K_{NBD-H}	k_{cat}/K_{NBD-H}	log ($1/K_{TS}$)	k_{cat}	$K_{NBD-ONH_2}$	$k_{cat}/K_{NBD-ONH_2}$	log ($1/K_{TS}$)	k_{cat}	$K_{NBD-ONH_2}$	$k_{cat}/K_{NBD-ONH_2}$	log ($1/K_{TS}$)
	($\times10^{-4}$ s^{-1})	($\times10^{-6}$ M)	(M^{-1} s^{-1})		($\times10^{-3}$ s^{-1})	($\times10^{-6}$ M)	(M^{-1} s^{-1})		($\times10^{-3}$ s^{-1})	($\times10^{-6}$ M)	(M^{-1} s^{-1})	
V15pAF	2.26 (0.11)	122 (12)	1.85 (0.10)	6.20	5.70 (0.32)	181 (24)	31.4 (4.7)	6.26	51.4 (2.90)	146 (15)	352 (19)	6.73
V15Y	0.90 (0.05)	538 (49)	0.17 (0.01)	ND	1.76 (0.21)	246 (49)	7.15 (0.73)	5.62	6.01 (0.41)	154 (19)	39.0 (2.50)	5.78
LmrR	2.20 (0.07)	239 (12)	0.92 (0.02)	ND	1.80 (0.14)	230 (29)	7.84 (0.42)	5.66	0.78 (0.03)	81 (8)	9.61 (0.58)	5.18

Catalytic parameters were obtained by fitting the data points from Figs. 3d, 4b and 4c to the Michaelis–Menten equation and are calculated per LmrR dimer. *Hydrazone formation reactions were performed at a 4-HBA concentration of 5 mM. †Oxime formation reactions were carried out in the presence of 250 μM 4-NBA. ‡In the absence of an available K_{is} for 4-NBA ($K_{4\text{-NBA}}$), apparent chemical proficiencies were obtained by substituting this term with the concentration of 4-NBA under the assay conditions (250 μM). As this concentration is well below the $K_{4\text{-HBA}}$ obtained from our studies for the hydrazone formation reaction, this estimate is likely to accurately reflect the transition-state stabilization. If the $K_{4\text{-HBA}}$ was significantly lower, the apparent $1/K_{TS}$ would underestimate the true value. 95% confidence intervals are shown in brackets. $K_{NBD-ONH_2}$: K_M for NBD-ONH_2; ND, not determined.

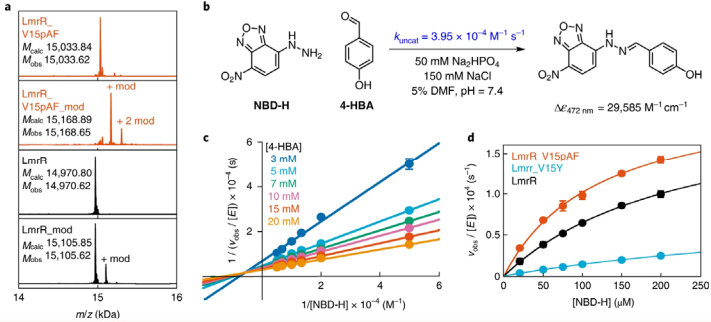
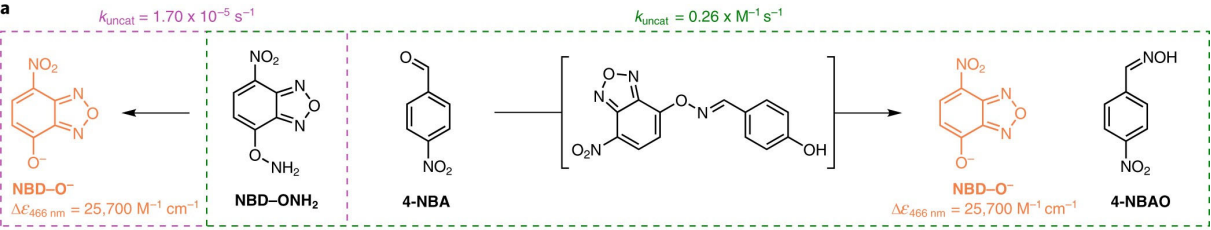
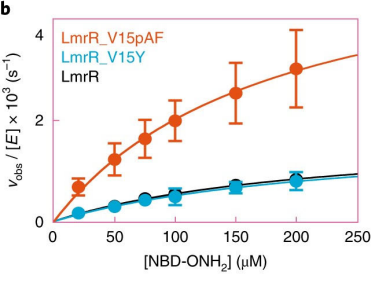


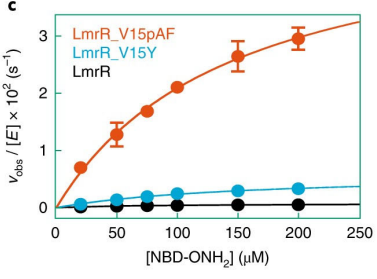
Table 1 | Catalytic parameters obtained from saturation kinetic studies for LmrR, LmrR_V15Y and LmrR_V15pAF

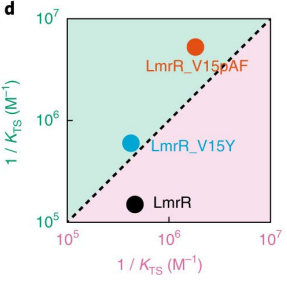
	Hydrazone formation*				NBD-ONH_2 hydrolysis				Oxime formation†			
	k_{cat}	K_{NBD-H}	k_{cat}/K_{NBD-H}	log ($1/K_{TS}$)	k_{cat}	$K_{NBD-ONH_2}$	$k_{cat}/K_{NBD-ONH_2}$	log ($1/K_{TS}$)	k_{cat}	$K_{NBD-ONH_2}$	$k_{cat}/K_{NBD-ONH_2}$	log ($1/K_{TS}$)
	($\times10^{-4}$ s^{-1})	($\times10^{-6}$ M)	(M^{-1} s^{-1})		($\times10^{-3}$ s^{-1})	($\times10^{-6}$ M)	(M^{-1} s^{-1})		($\times10^{-3}$ s^{-1})	($\times10^{-6}$ M)	(M^{-1} s^{-1})	
V15pAF	2.26 (0.11)	122 (12)	1.85 (0.10)	6.20	5.70 (0.32)	181 (24)	31.4 (4.7)	6.26	51.4 (2.90)	146 (15)	352 (19)	6.73
V15Y	0.90 (0.05)	538 (49)	0.17 (0.01)	ND	1.76 (0.21)	246 (49)	7.15 (0.73)	5.62	6.01 (0.41)	154 (19)	39.0 (2.50)	5.78
LmrR	2.20 (0.07)	239 (12)	0.92 (0.02)	ND	1.80 (0.14)	230 (29)	7.84 (0.42)	5.66	0.78 (0.03)	81 (8)	9.61 (0.58)	5.18

Catalytic parameters were obtained by fitting the data points from Figs. 3d, 4b and 4c to the Michaelis–Menten equation and are calculated per LmrR dimer. *Hydrazone formation reactions were performed at a 4-HBA concentration of 5 mM. †Oxime formation reactions were carried out in the presence of 250 μM 4-NBA. ‡In the absence of an available K_{is} for 4-HBA ($K_{4\text{-NBA}}$), apparent chemical proficiencies were obtained by substituting this term with the concentration of 4-NBA under the assay conditions (250 μM). As this concentration is well below the $K_{4\text{-HBA}}$ obtained from our studies for the hydrazone formation reaction, this estimate is likely to accurately reflect the transition-state stabilization. If the $K_{4\text{-HBA}}$ was significantly lower, the apparent $1/K_{TS}$ would underestimate the true value. 95% confidence intervals are shown in brackets. $K_{NBD-ONH_2}$: K_M for NBD-ONH_2; ND, not determined.


a
k_{uncat} = 1.70 x 10^{-5} s^{-1}
k_{uncat} = 0.26 x M^{-1} s^{-1}
NBD–O⁻
$\Delta\varepsilon_{466\ nm}$ = 25,700 M^{-1} cm^{-1}
NBD–ONH₂
4-NBA
NBD–O⁻
$\Delta\varepsilon_{466\ nm}$ = 25,700 M^{-1} cm^{-1}
4-NBAO


b
LmrR_V15pAF
LmrR_V15Y
LmrR
v_{obs} / [E] x 10^3 (s^{-1})
[NBD-ONH₂] (μM)


c
LmrR_V15pAF
LmrR_V15Y
LmrR
v_{obs} / [E] x 10^2 (s^{-1})
[NBD-ONH₂] (μM)


d
LmrR_V15pAF
LmrR_V15Y
LmrR
1 / K_{TS} (M^{-1})
1 / K_{TS} (M^{-1})

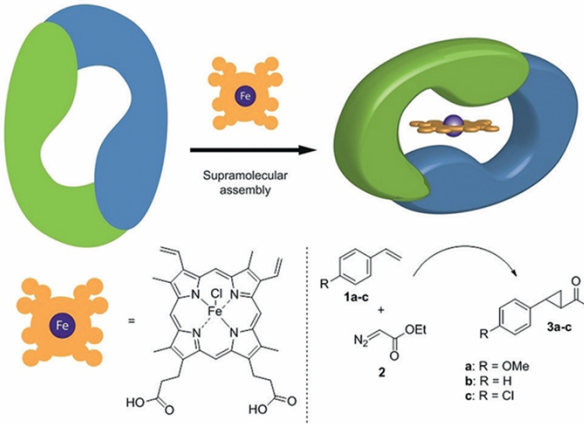


Table 1: Results of cyclopropanation reaction of styrene derivatives **1a–c** with EDA (**2**) catalyzed by LmrR⊂heme.[a]

entry	LmrR	1[c]	3	Yield [%]	TTN	3/4	*ee* [%][b]
1	–	**1a**	**3a**	5±2	51	0.8	–
2	LmrR	**1a**	**3a**	25±11	247	11	17±5
3	LmrR_F93A	**1a**	**3a**	23±2	232	9	11±1
4	LmrR_D100A	**1a**	**3a**	38±8	375	20	24±5
5	LmrR_W96A	**1a**	**3a**	28±13	276	8	<5
6	LmrR_V15A	**1a**	**3a**	1.5±0.5	15	3	17±1
7	LmrR_M8A	**1a**	**3a**	36±13	359	15	44±12
8[c]	LmrR_M8A	**1a**	**3a**	45±9	449	6	51±14
9[c]	–	**1b**	**3b**	6±0	59	n.d	–
10[c]	LmrR_M8A	**1b**	**3b**	39±13	391	n.d	38±5[d]
11[c]	–	**1c**	**3c**	1±0	12	0.2	–
12[c]	LmrR_M8A	**1c**	**3c**	35±13	351	3	25±5

[a] Conditions: 1 (30 mM), 2 (10 mM), hemin (1 mol%; 10 μM), LmrR_X (1.1 mol%; 11 μM) in 50 mM phosphate buffer (pH 8.0), under Ar, at 4°C for 18 h; Results are the average of at least two independent experiments, both carried out in duplicate. [b] *ee* of the *trans* product; *trans*/*cis* > 85:15. [c] pH 7.0. [d] *ee* of the 1*R*, 2*R* enantiomer.[37]

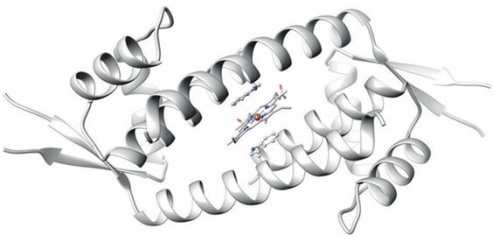


Figure 2. Crystal structure of LmrR⊂heme (PDB ID: 6FUU). The protein crystallized in a tetragonal crystal form with one polypeptide chain occupying the asymmetric unit; the functional dimer (here shown in cartoon representation) is formed by a crystallographic dyad. In the electron density maps, the polypeptide chain is well defined, except for the tip region of the β-wing (residues 70–73) and the N- and C-termini (residues 1–4 and 109–131, including the C-terminal strep-tag). These regions show a high degree of disorder and were excluded from the final model. The heme is stacked in between the side chains of W96/W96′ (for clarity only one of the alternate heme binding orientations is shown).

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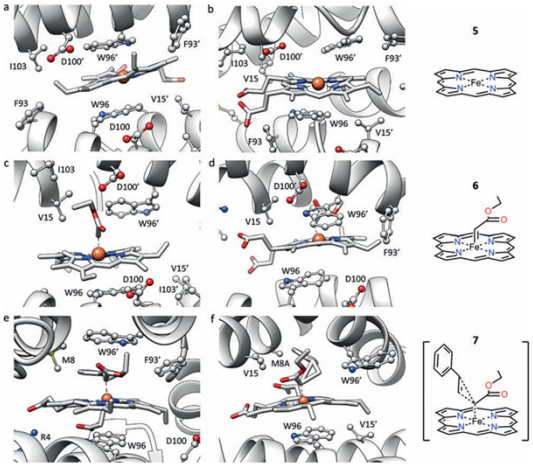
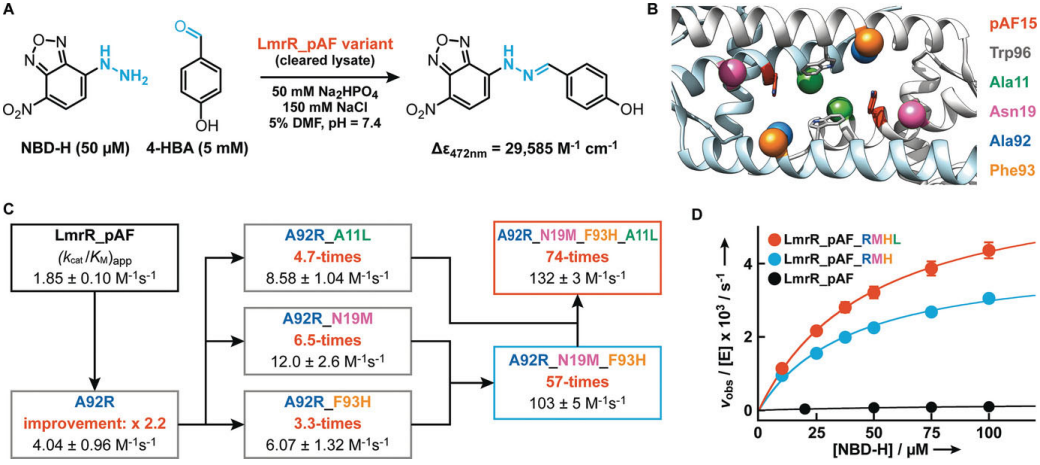


Figure 3. Representative structures resulting from MD simulations. a, b) The LmrR-heme system (cluster 3, RMSD with crystal 1.634 Å (a) and cluster 2 RMSD with crystal 2.471 Å (b); 400 ns MD simulation). c, d) The LmrR–heme–carbene system (cluster 0, RMSD with crystal of 0.548 Å (c) and cluster 2, RMSD with crystal of 1.000 Å (d); 400 ns MD simulation). e, f) The cyclopropanation transition state (with LmrR (e) and with LmrR_M8A (f); 100 ns MD simulation).


A
LmrR_pAF variant
(cleared lysate)
50 mM Na2HPO4
150 mM NaCl
5% DMF, pH = 7.4
NBD-H (50 µM)
4-HBA (5 mM)
Δε472nm = 29,585 M-1 cm-1
B
pAF15
Trp96
Ala11
Asn19
Ala92
Phe93
C
LmrR_pAF
(kcat/KM)app
1.85 ± 0.10 M-1s-1
A92R
improvement: x 2.2
4.04 ± 0.96 M-1s-1
A92R_A11L
4.7-times
8.58 ± 1.04 M-1s-1
A92R_N19M
6.5-times
12.0 ± 2.6 M-1s-1
A92R_F93H
3.3-times
6.07 ± 1.32 M-1s-1
A92R_N19M_F93H_A11L
74-times
132 ± 3 M-1s-1
A92R_N19M_F93H
57-times
103 ± 5 M-1s-1
D
LmrR_pAF_RMHL
LmrR_pAF_RMH
LmrR_pAF
vObs / [E] x 10³ / s-1
[NBD-H] / µM
0
25
50
75
100
2
4

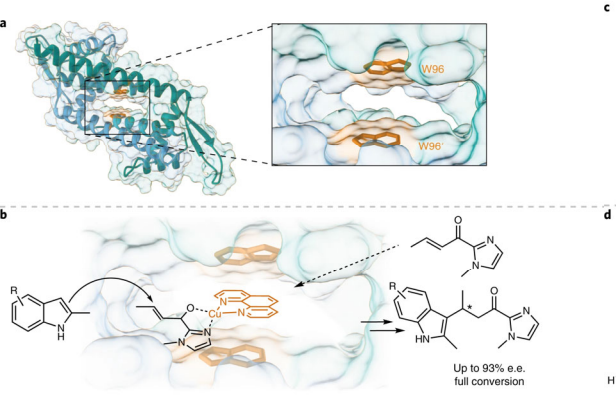


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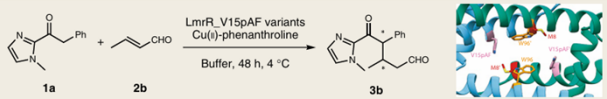
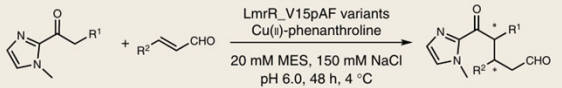
Table 2 | Optimization of reaction conditions and mutagenesis study for artificial enzymes



Entry	Catalysts		Yield (%)[a]	d.r.[b]	e.e. (%)[b]
1	LmrR	Cu(II)-phen	<1	n.d.	n.d.
2[c]	LmrR + aniline	Cu(II)-phen	<1	n.d.	n.d.
3	LmrR_V15pAF	Cu(II)-phen	14 ± 3	1:1	57 ± 2/39 ± 1
4	LmrR_V15pAF	$Cu(NO_3)_2$	54 ± 2	3.5:1	98 ± 0/86 ± 2
5[c]	LmrR_V15pAF	Cu(II)-phen	65 ± 1	4:1	98 ± 0/86 ± 1
6[d]	LmrR_V15pAF	Cu(II)-phen	40 ± 2	4:1	98 ± 0/84 ± 1
7[e]	LmrR_V15pAF	Cu(II)-phen	15 ± 1	4:1	98 ± 0/84 ± 0
8[f]	LmrR_V15pAF	Cu(II)-phen	6 ± 1	4:1	99 ± 0/77 ± 1
9[c]	LmrR_V15pAF_W96A	Cu(II)-phen	12 ± 2	1.2:1	82 ± 1/49 ± 2
10[c]	LmrR_V15pAF_M8R	Cu(II)-phen	34 ± 3	4:1	98 ± 0/81 ± 1
11[c]	LmrR_V15pAF_M8D	Cu(II)-phen	61 ± 1	3:1	96 ± 0/62 ± 2
12[c]	LmrR_V15pAF_M8W	Cu(II)-phen	64 ± 3	7:1	99 ± 0/84 ± 2
13[c]	LmrR_V15pAF_M8I	Cu(II)-phen	76 ± 2	6:1	>99 ± 0/88 ± 1
14[c]	LmrR_V15pAF_M8L	Cu(II)-phen	82 ± 1	6:1	>99 ± 0/93 ± 1
15[d]	LmrR_V15pAF_M8L	Cu(II)-phen	68 ± 2	6:1	>99 ± 0/93 ± 1
16[e]	LmrR_V15pAF_M8L	Cu(II)-phen	36 ± 1	5.4:1	>99 ± 0/92 ± 0
17[f]	LmrR_V15pAF_M8L	Cu(II)-phen	15 ± 2	5:1	>99 ± 0/90 ± 0

Typical conditions: 0.8 equiv. Cu(II)-phen (4 mol%; 40 μM) loading with respect to LmrR, LmrR_V15pAF or variants (5 mol%; 50 μM), 1 mM **1a**, 10 mM **2b**, in 20 mM MOPS buffer (pH 7.0), 150 mM NaCl, at 4 °C for 48 h, unless noted otherwise. Yield and e.e. values are the average of at least two independent experiments, both carried out in duplicate. All error values are given as s.d. [a]Yields were determined by HPLC. [b]e.e. and diastereomeric ratio (d.r.) values were determined by chiral HPLC. n.d., not determined. [c]LmrR (5 mol%; 50 μM), aniline (5 mol%; 50 μM) and Cu(II)-phen (5 mol%; 50 μM). [d]Conditions: 1.2 equiv. Cu(II)-phen (6 mol%; 60 μM) loading with respect to LmrR_V15pAF or variants (5 mol%; 50 μM), 1 mM **1a**, 10 mM **2b**, in 20 mM MES buffer (pH 6.0), 150 mM NaCl, at 4 °C for 48 h. [e]Reaction with 2.5 mol% protein and 3 mol% Cu(II)-phen loading. [f]Reaction with 1 mol% protein and 1.2 mol% Cu(II)-phen loading. [g]Reaction with 0.5 mol% protein and 0.6 mol% Cu(II)-phen loading. The image in the top-right is a close-up of the hydrophobic pore in LmrR_pAF crystal structure (PDB: 6I8N). Catalytic aniline side chains (pink), Trp96 (yellow) and Met8 (red) are shown as sticks. n.d., not determined.

Table 3 | Substrate scope of Michael addition reactions catalysed by LmrR-based artificial enzymes



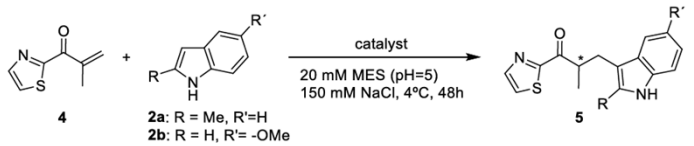
1 2 3a–h

a: R^1=Ph, R^2=H **e**: R^1=*p*-MeOPh, R^2=CH_3
b: R^1=Ph, R^2=CH_3 **f**: R^1=*p*-MeOPh, R^2=H
c: R^1=Ph, R^2=*n*Pr **g**: R^1=*p*-Cl, R^2=CH_3
d: R^1=Ph, R^2=Ph **h**: R^1=3-thienyl, R^2=CH_3

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

Typical conditions: 1.2 equiv. Cu(II)-phen (6 mol%; 60 μM) loading with respect to LmrR_V15pAF and LmrR_V15pAF_M8L (5 mol%; 50 μM), 1 mM **1**, 10 mM **2**, in 20 mM MES buffer (pH 6.0), 150 mM NaCl, at 4 °C for 48 h, unless noted otherwise. Yield and e.e. values are the average of at least two independent experiments, both carried out in duplicate. All error values are given as s.d. [a]Yields were determined by HPLC. [b]e.e. and d.r. values were determined by chiral HPLC.

Table 1. Results of the Tandem Friedel-Crafts/Enantioselective Protonation (FC/EP Reaction) of Indoles with 4[a]



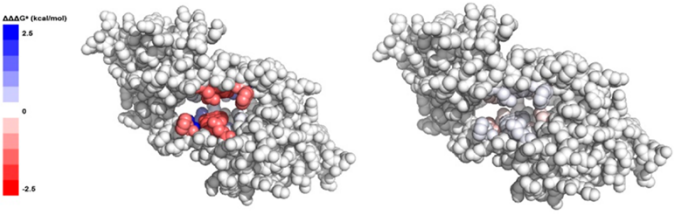
entry	indole	product	catalyst (μM)	yield of 5 (%)	ee (%)[b]
1	2b	5b	$Cu(NO_3)_2$	<5	
2	2b	5b	Cu(II)-phen	<5	
3	2b	5b	LmrR	10 ± 1	61 ± 2
4	2b	5b	$Cu(NO_3)_2$ + LmrR	86 ± 10	<5
5	2b	5b	Cu(II)-phen + LmrR	58 ± 12	−40 ± 2
6	2a	5a	Cu(II)-phen + LmrR	87 ± 14	59 ± 3

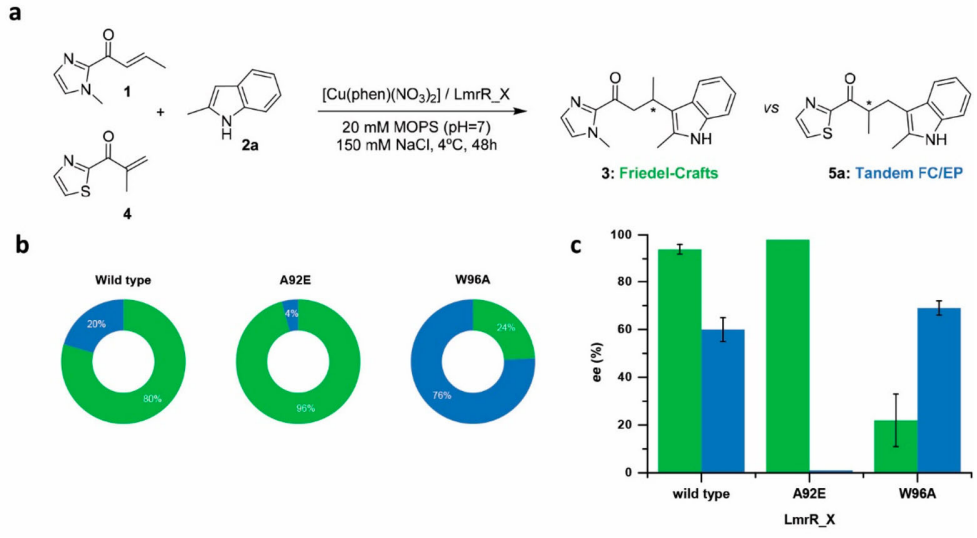
[a]Typical reaction conditions: **4** (1 mM), **2a**/**b** (1 mM) in 20 mM MES buffer (pH 5.0), 150 mM NaCl, at 4 °C for 72h; results are the average of at least two independent experiments, both carried out in duplicate. Error margins represent standard deviations. [b]determined by hplc; + and−signs indicate which is the major enantiomer peak based on order of elution, first and second, respectively.

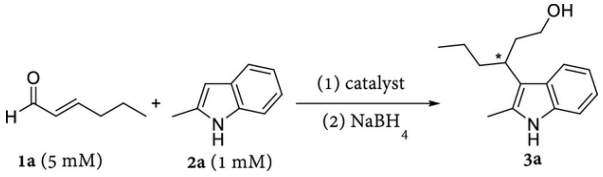
a

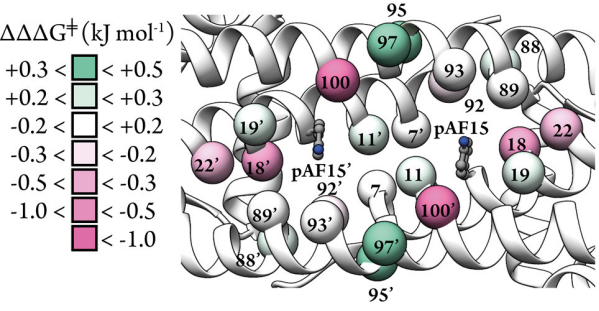
Mutant:	ee (%) FC	ee (%) FC/EP
D100A	46 ± 4	63 ± 1
F93A	51 ± 5	66 ± 2
A92E	> 99	21 ± 7
W96A	25 ± 9	64 ± 6
Q12A	98 ± 1	44 ± 6
V99A	95 ± 1	60 ± 4
M8A	99 ± 0	45 ± 1
V15A	94 ± 1	42 ± 1
E7A	96 ± 1	56 ± 1

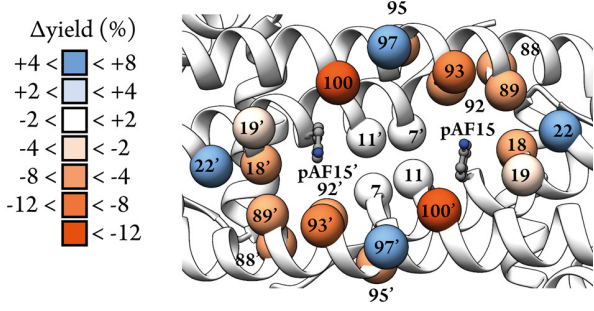
b

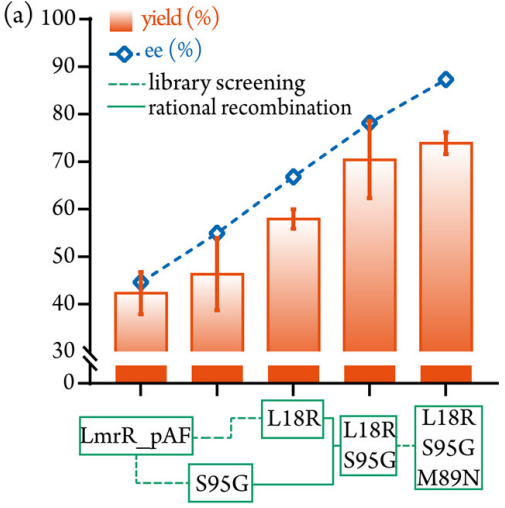



a
1
4
2a
[Cu(phen)(NO3)2] / LmrR_X
20 mM MOPS (pH=7)
150 mM NaCl, 4ºC, 48h
3: Friedel-Crafts
vs
5a: Tandem FC/EP
b
Wild type
20%
80%
A92E
4%
96%
W96A
24%
76%
c
ee (%)
100
80
60
40
20
0
wild type
A92E
W96A
LmrR_X


O
H
+
N
H
(1) catalyst
(2) NaBH4
OH
*
N
H
1a (5 mM)
2a (1 mM)
3a


ΔΔΔG‡ (kJ mol-1)
+0.3 < < +0.5
+0.2 < < +0.3
-0.2 < < +0.2
-0.3 < < -0.2
-0.5 < < -0.3
-1.0 < < -0.5
< -1.0
95
97
88
93
89
100
92
19'
11'
7'
pAF15
22
18
22'
18'
pAF15'
92'
11
19
7
89'
93'
100'
97'
88'
95'


Δyield (%)
+4 < < +8
+2 < < +4
-2 < < +2
-4 < < -2
-8 < < -4
-12 < < -8
< -12
95
97
88
93
89
100
92
19'
11'
7'
pAF15
22
18
22'
18'
pAF15'
92'
11
19
7
89'
93'
100'
97'
88'
95'


(a)
100
90
80
70
60
50
40
30
0
yield (%)
ee (%)
library screening
rational recombination
LmrR_pAF
L18R
S95G
L18R
S95G
L18R
S95G
M89N

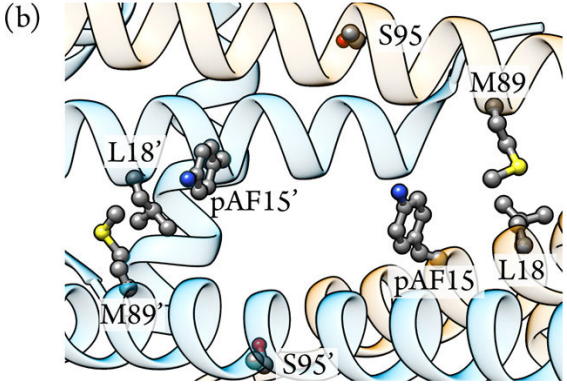
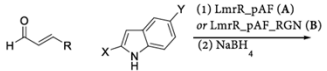

(b)
S95
M89
L18'
pAF15'
pAF15
L18
M89'
S95'

Table 2. Substrate Scope of Indoles and Enals Converted to the Corresponding Friedel–Crafts Products 3a–3i by LmrR_pAF and LmrR_pAF_RGN[a]



3a X = Me, Y = H, R = *n*-Pr
3b X = Me, Y = H, R = *i*-Pr
3c X = Me, Y = H, R = Me
3d X = Me, Y = H, R = Ph
3e X = H, Y = H, R = *n*-Pr
3f X = H, Y = Me, R = *n*-Pr
3g X = H, Y = OMe, R = *n*-Pr
3h X = H, Y = Br, R = *n*-Pr
3i X = Me, Y = OMe, R = *n*-Pr

[a]Reaction conditions: LmrR variants (20 μM dimer concentration) in pH 6.5 buffer containing NaCl (150 mM) and NaH_2PO_4 (50 mM), 8 vol % DMF, [indole] = 1 mM, [enal] = 5 mM, reaction time 16 h, reactions conducted at 4 °C with mixing by continuous inversion in 300 μL total volume. Reduction with $NaBH_4$ (60 μL, 20 mg mL^{-1} in 0.5 w/v% NaOH) afforded the alcohol products **3a–3i**. For each entry, two independent experiments were conducted, each in duplicate. Errors are the standard deviation of the results thus obtained. [b]Analytical yields determined using normal phase HPLC using 3-(3-hydroxypropyl)indole as internal standard. [c]Enantiomeric excess determined using chiral normal-phase HPLC (Chiracel OJ-H (**3a**), AS-H (**3b**, **3c**, **3f**), OD-H (**3d**, **3e**, **3h**, **3i**, **3j**) or OB-H (**3g**)). [d]Reaction time 40 h. [e]LmrR was used in place of LmrR_pAF. [f]No product could be detected. [g]50 μM artificial enzyme (concentration of dimer) was used, and reaction time was 64 h. [h]Absolute configuration asigned by comparison of order of elution on chiral normal-phase HPLC with enantioenriched reference compound and the literature;[18] see Supporting Information for details.

entry	product	catalyst	yield (%)[b]	ee (%)[c]
1	**3a**	A	42 ± 4	45 ± 0
2	**3a**	B	74 ± 2	87 ± 0
3[d]	**3b**	A	24 ± 1	58 ± 0
4[d]	**3b**	B	34 ± 6	83 ± 2
5	**3c**	A	76 ± 7	33 ± 1
6	**3c**	B	95 ± 7	58 ± 1
7	**3d**	A	3 ± 0	20 ± 1
8	**3d**	B	4 ± 1	13 ± 9
9[e,f]	**3d**	LmrR	<1	-
10[g]	**3e**	A	10 ± 1	50 ± 0
11[g]	**3e**	B	27 ± 2	89 ± 1
12[g]	**3f**	A	16 ± 1	41 ± 0
13[g]	**3f**	B	31 ± 3	89 ± 2
14[g]	**3g**	A	24 ± 2	55 ± 1
15[g]	**3g**	B	40 ± 3	89 ± 1
16[f]	**3h**	A	<1	-
17[f]	**3h**	B	<1	-
18	**3i**	A	62 ± 11	58 ± 0
19	**3i**	B	75 ± 9	82 ± 1
20[g]	**3j**	A	21 ± 1	66 ± 0 (S)[h]
21[g]	**3j**	B	18 ± 2	73 ± 1 (S)[h]

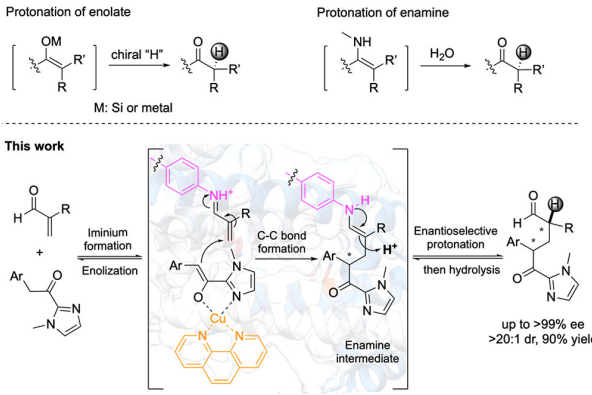
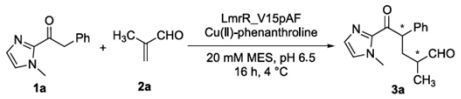


Table 1. Results of the LmrR_V15pAF/Cu(II)-phen Catalyzed Tandem Michael Addition/Enantioselective Protonation Reactions[a]

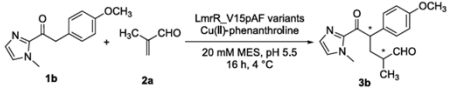


	catalyst				
entry			yield (%)[b]	dr[c]	ee (%)[c,d]
1	LmrR_pAF		<1	ND	ND
2		Cu(II)-phen	<1	ND	ND
3	LmrR	Cu(II)-phen	<1	ND	ND
4	LmrR_pAF	Cu(II)-phen	26 ± 3	3:1	89 ± 0/32 ± 1
5[e]	LmrR_pAF	Cu(II)-phen	14 ± 2	6:1	93 ± 0/24 ± 2

[a]Conditions used: LmrR_V15pAF or LmrR (5 mol %; 50 μM), 1.2 equiv of Cu(II)-phen (6 mol %; 60 μM), **1a** (1 mM), **2a** (10 mM), in MES buffer (20 mM, pH 6.5), NaCl (150 mM), at 4 °C for 48 h, unless noted otherwise. Yields and ee values are the averages of independent experiments, performed at least in triplicate. [b]Determined by HPLC analysis. [c]dr and ee values were determined by chiral HPLC. [d]ee values of major diastereomer. [e]Reactions carried out for 16 h. Errors represent standard deviations based on at least three independent experiments. ND = not determined.

ACS Catal. **2021**, *11*, 9366−9369

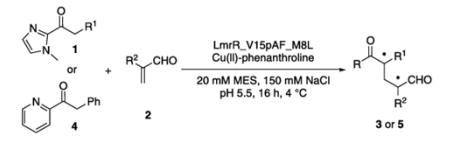
Table 2. Study of Reaction Conditions and Effect of LmrR Mutations[a]

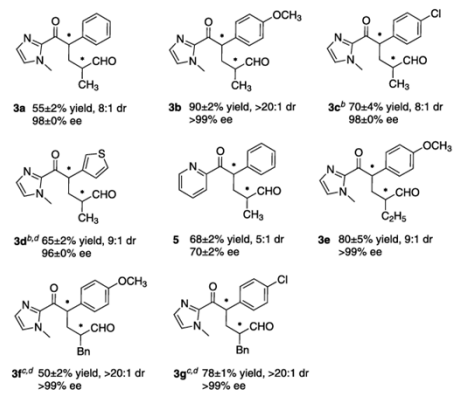


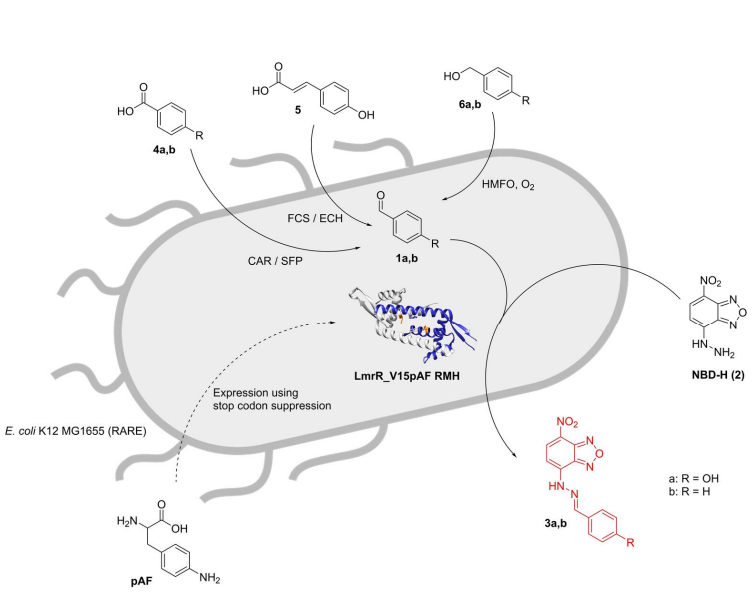
entry	enzyme + Cu(II)-phen	yield (%)[b]	dr[c]	ee (%)[c,d]
1[e]	LmrR_V15pAF	44 ± 2	10:1	99 ± 0
2	LmrR_V15pAF	28 ± 3	10:1	99 ± 0
3[f]	LmrR_V15pAF	16 ± 1	9:1	99 ± 0
4	LmrR_V15pAF_W96A	5 ± 1	3:1	18 ± 1
5	LmrR_V15pAF_M8W	40 ± 1	15:1	99 ± 0
6	LmrR_V15pAF_M8I	58 ± 3	18:1	>99
7	LmrR_V15pAF_M8L	65 ± 2	20:1	>99
8[g]	LmrR_V15pAF_M8L	33 ± 2	5:1	99 ± 0
9[h]	LmrR_V15pAF_M8L	88 ± 1	8:1	99 ± 0
10[e]	LmrR_V15pAF_M8L	90 ± 2	>20:1	>99

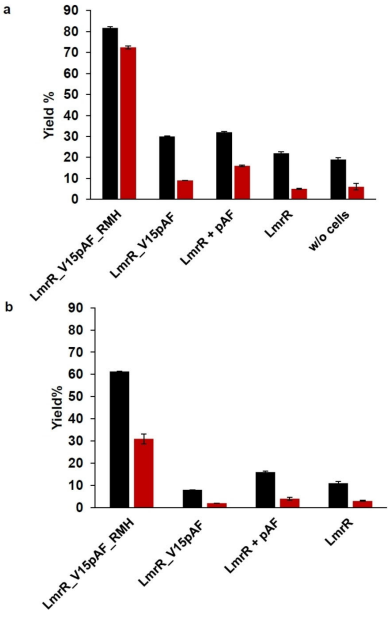
[a]Conditions used: LmrR_V15pAF or mutants (2.5 mol %; 25 μM), 1.2 equiv pf Cu(II)-phen (3 mol %; 30 μM), **1b** (1 mM), **2a** (10 mM), in MES buffer (20 mM, pH 5.5), NaCl (150 mM), at 4 °C for 16 h, unless noted otherwise. Yields and ee values are the average of independent experiments, performed at least in triplicate. [b]Determined by HPLC. [c]dr and ee values were determined by chiral HPLC. [d]ee values for major/minor diastereomer. In cases where the dr was high, the ee of the minor diastereomer was not determined. [e]5 mol % of LmrR_V15pAF variant and 6 mol % of Cu(II)-phen. [f]1 mol % of LmrR_V15pAF and 1.2 mol % of Cu(II)-phen. [g]Reaction performed at 18 °C. [h]Reaction performed for 48 h. Errors represent standard deviation based on independent experiments performed at least in triplicate. The ee of the minor diastereomers are not shown in this table but in Table S2 of the Supporting Information.

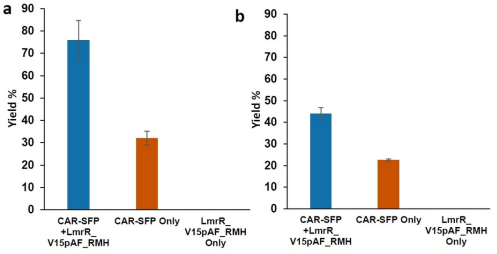
Table 3. Substrate Scope[a]

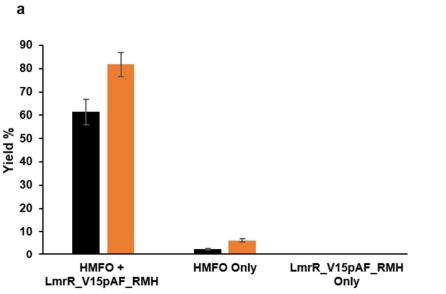


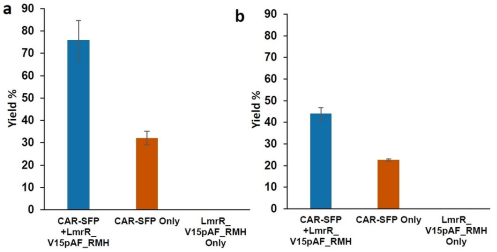


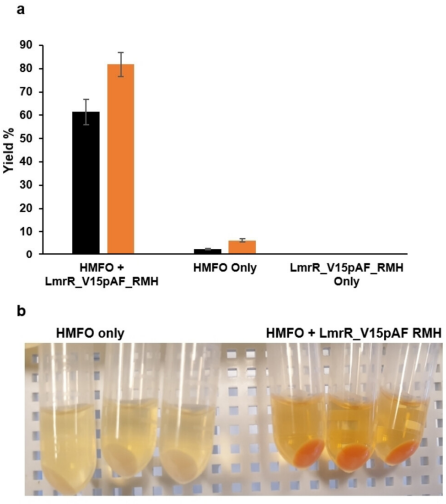

4a,b
5
6a,b
HMFO, O2
FCS / ECH
CAR / SFP
1a,b
LmrR_V15pAF RMH
NBD-H (2)
Expression using
stop codon suppression
E. coli K12 MG1655 (RARE)
pAF
3a,b
a: R = OH
b: R = H

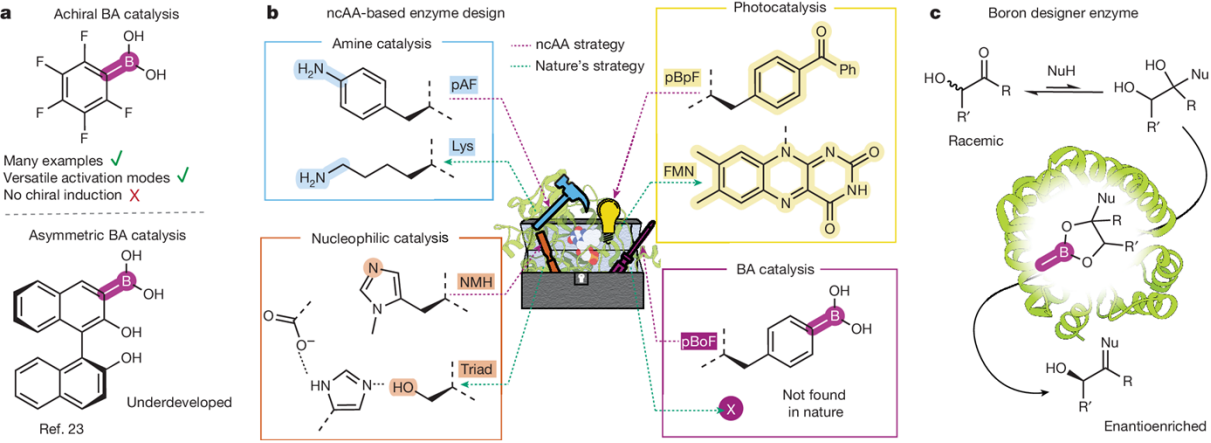

a
Yield %
LmrR_V15pAF_RMH
LmrR_V15pAF
LmrR + pAF
LmrR
w/o cells
b
Yield%
LmrR_V15pAF_RMH
LmrR_V15pAF
LmrR + pAF
LmrR

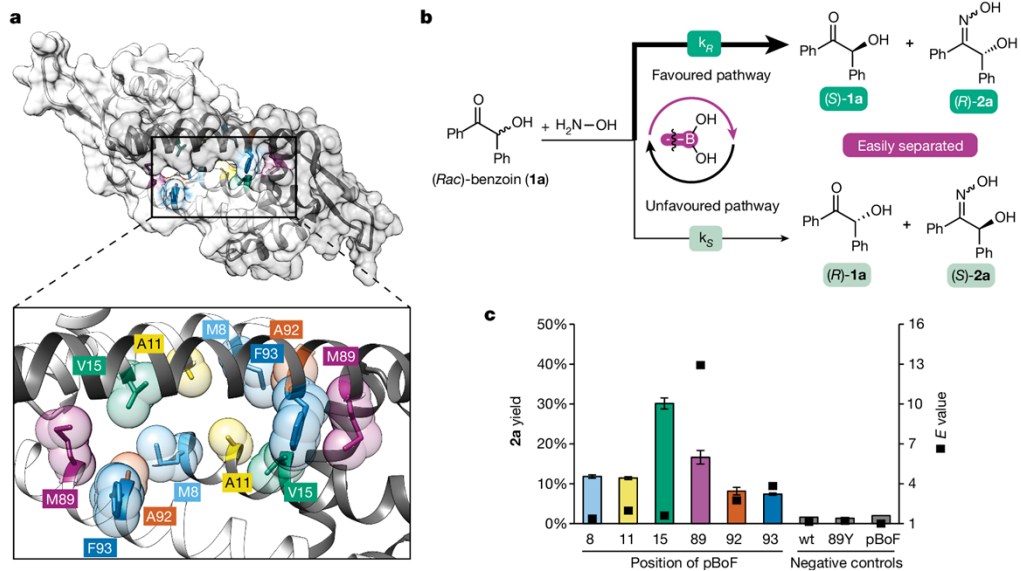

a
b
c
Yield %
CAR-SFP +LmrR_ V15pAF_RMH
CAR-SFP Only
LmrR_ V15pAF_RMH Only
FCS-ECH +LmrR_ V15pAF_RMH
FCS-ECH Only

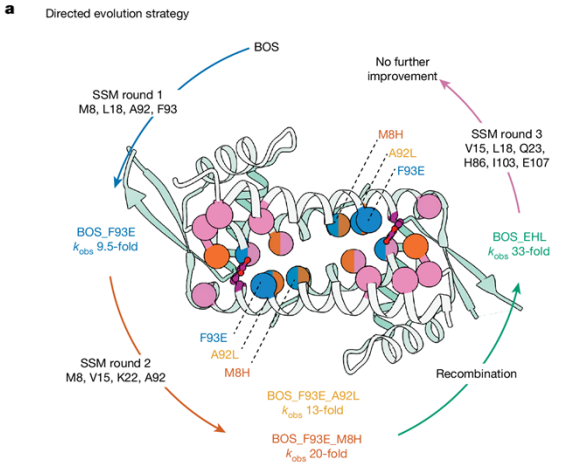

a
b
Yield %
HMFO + LmrR_V15pAF_RMH
HMFO Only
LmrR_V15pAF_RMH Only
HMFO only
HMFO + LmrR_V15pAF RMH

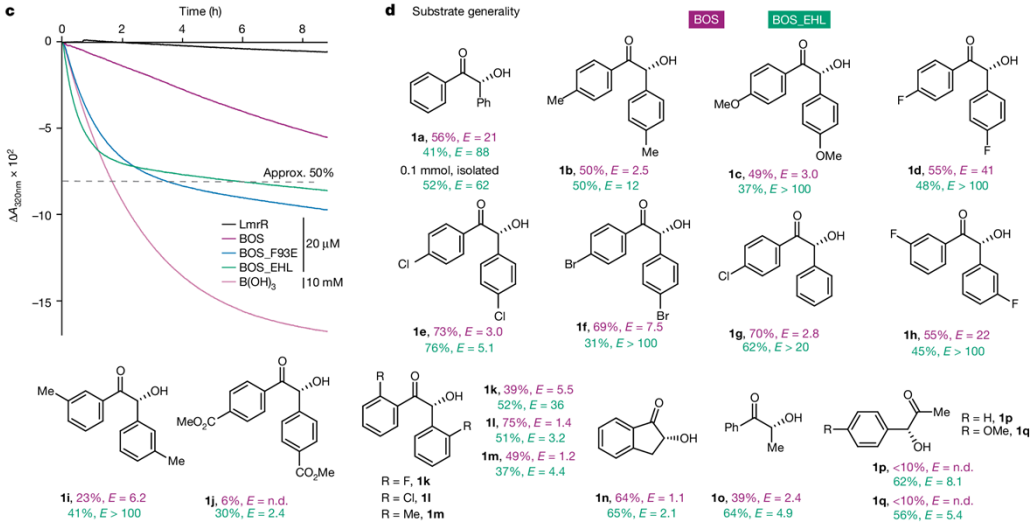

a
Yield %
CAR-SFP +LmrR_ V15pAF_RMH
CAR-SFP Only
LmrR_ V15pAF_RMH Only
b
Yield %
CAR-SFP +LmrR_ V15pAF_RMH
CAR-SFP Only
LmrR_ V15pAF_RMH Only
c
Yield %
FCS-ECH +LmrR_ V15pAF_RMH
FCS-ECH Only
LmrR_ V15pAF_RMH Only

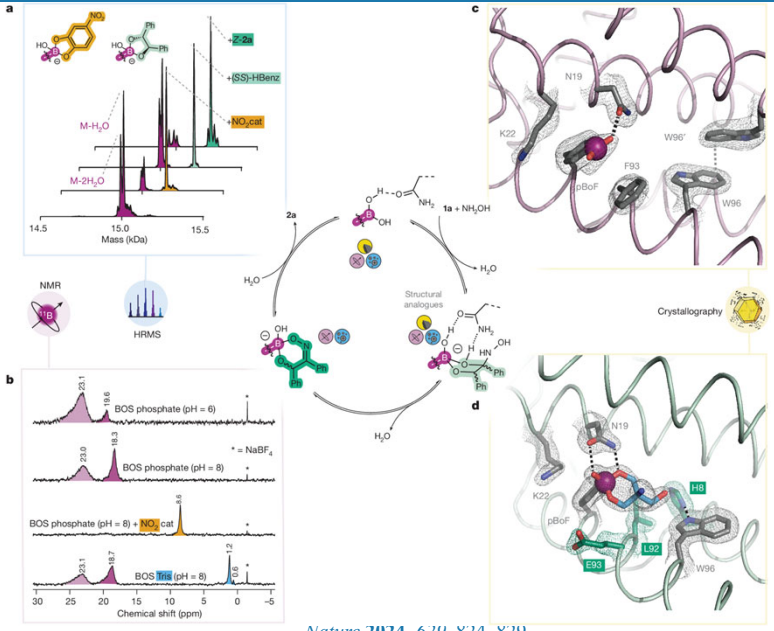

a
Yield %
HMFO + LmrR_V15pAF_RMH
HMFO Only
LmrR_V15pAF_RMH Only
b
HMFO only
HMFO + LmrR_V15pAF RMH

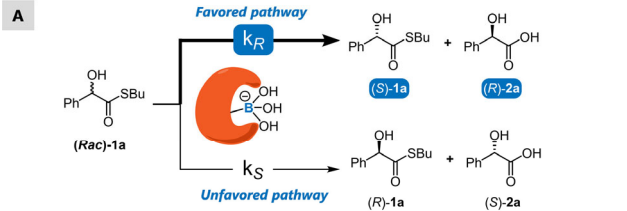

a
Achiral BA catalysis
Many examples ✓
Versatile activation modes ✓
No chiral induction X
Asymmetric BA catalysis
Underdeveloped
Ref. 23
b
ncAA-based enzyme design
ncAA strategy
Nature's strategy
Amine catalysis
pAF
Lys
Nucleophilic catalysis
NMH
Triad
Photocatalysis
pBpF
FMN
BA catalysis
pBoF
Not found in nature
c
Boron designer enzyme
NuH
Racemic
Enantioenriched

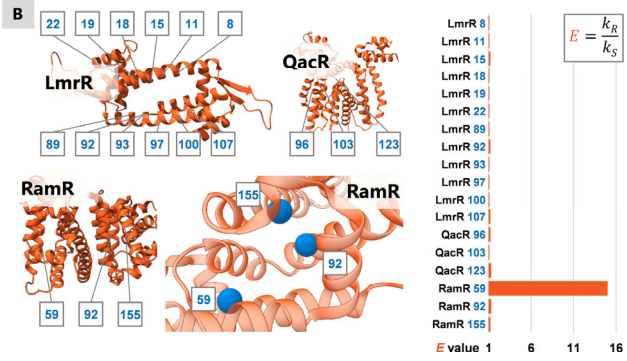

a
V15
A11
M8
A92
F93
M89
M89
M8
A11
V15
A92
F93
b
(Rac)-benzoin (1a)
+ H2N–OH
kR
Favoured pathway
(S)-1a
(R)-2a
Easily separated
Unfavoured pathway
kS
(R)-1a
(S)-2a
c
2a yield
E value
50%
40%
30%
20%
10%
0%
16
13
10
7
4
1
8
11
15
89
92
93
wt
89Y
pBoF
Position of pBoF
Negative controls

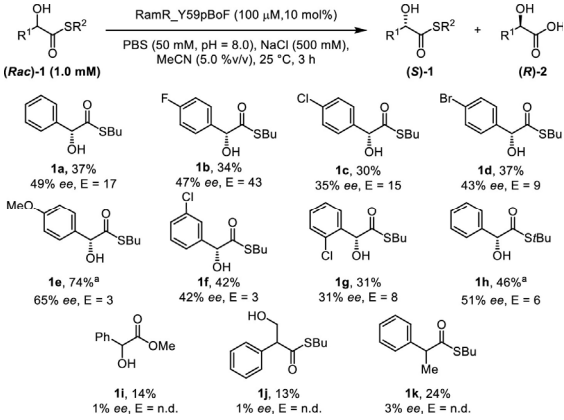

a
Directed evolution strategy
BOS
SSM round 1
M8, L18, A92, F93
BOS_F93E
k_{obs} 9.5-fold
SSM round 2
M8, V15, K22, A92
F93E
A92L
M8H
BOS_F93E_A92L
k_{obs} 13-fold
BOS_F93E_M8H
k_{obs} 20-fold
Recombination
BOS_EHL
k_{obs} 33-fold
SSM round 3
V15, L18, Q23,
H86, I103, E107
No further
improvement
M8H
A92L
F93E
b
k_{obs} (h^{-1})
E value
BOS
F93E
A92L
M8H
A92L/M8H
BOS_EHL

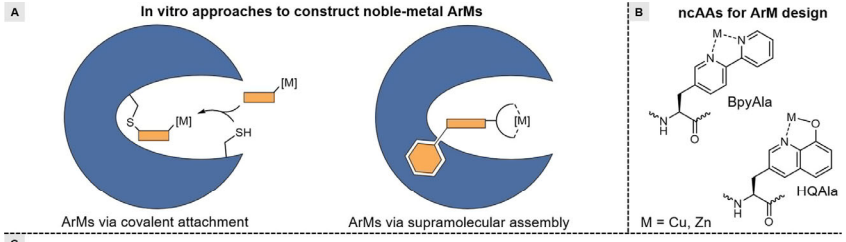

c
Time (h)
0
2
4
6
8
0
−5
−10
−15
ΔA320nm × 10^6
Approx. 50%
LmrR
BOS
BOS_F93E
BOS_EHL
B(OH)3
20 μM
10 mM
d
Substrate generality
BOS
BOS_EHL
1a, 56%, E = 21
41%, E = 88
0.1 mmol, isolated
52%, E = 62
1b, 50%, E = 2.5
50%, E = 12
1c, 49%, E = 3.0
37%, E > 100
1d, 55%, E = 41
48%, E > 100
1e, 73%, E = 3.0
76%, E = 5.1
1f, 69%, E = 7.5
31%, E > 100
1g, 70%, E = 2.8
62%, E > 20
1h, 55%, E = 22
45%, E > 100
1i, 23%, E = 6.2
41%, E > 100
1j, 6%, E = n.d.
30%, E = 2.4
R = F, 1k
R = Cl, 1l
R = Me, 1m
1k, 39%, E = 5.5
52%, E = 36
1l, 75%, E = 1.4
51%, E = 3.2
1m, 49%, E = 1.2
37%, E = 4.4
1n, 64%, E = 1.1
65%, E = 2.1
1o, 39%, E = 2.4
64%, E = 4.9
R = H, 1p
R = OMe, 1q
1p, <10%, E = n.d.
62%, E = 8.1
1q, <10%, E = n.d.
56%, E = 5.4

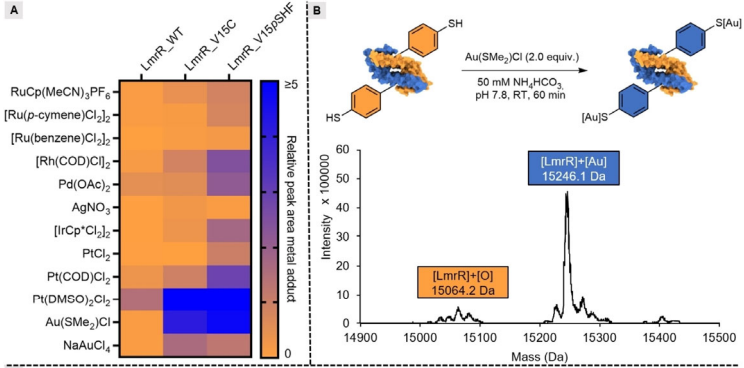

a
2-2a
(SS)-HBenz
NO2cat
M-H2O
M-2H2O
14.5
15.0
15.5
Mass (kDa)
NMR
HRMS
b
BOS phosphate (pH = 6)
BOS phosphate (pH = 8)
* = NaBF4
BOS phosphate (pH = 8) + NO2 cat
BOS Tris (pH = 8)
Chemical shift (ppm)
2a
1a + NH2OH
H2O
Structural analogues
c
N19
K22
W96'
F93
pBoF
W96
Crystallography
d
N19
K22
pBoF
H8
L92
E93
W96


A
Favored pathway
k_R
(Rac)-1a
(S)-1a
(R)-2a
k_S
Unfavored pathway
(R)-1a
(S)-2a


B
LmrR
QacR
RamR
RamR
LmrR 8
LmrR 11
LmrR 15
LmrR 18
LmrR 19
LmrR 22
LmrR 89
LmrR 92
LmrR 93
LmrR 97
LmrR 100
LmrR 107
QacR 96
QacR 103
QacR 123
RamR 59
RamR 92
RamR 155
E value 1
6
11
16
$E = \frac{k_R}{k_S}$


RamR_Y59pBoF (100 μM,10 mol%)
PBS (50 mM, pH = 8.0), NaCl (500 mM),
MeCN (5.0 %v/v), 25 °C, 3 h
(Rac)-1 (1.0 mM)
(S)-1
(R)-2
1a, 37%
49% ee, E = 17
1b, 34%
47% ee, E = 43
1c, 30%
35% ee, E = 15
1d, 37%
43% ee, E = 9
1e, 74%a
65% ee, E = 3
1f, 42%
42% ee, E = 3
1g, 31%
31% ee, E = 8
1h, 46%a
51% ee, E = 6
1i, 14%
1% ee, E = n.d.
1j, 13%
1% ee, E = n.d.
1k, 24%
3% ee, E = n.d.


A
In vitro approaches to construct noble-metal ArMs
[M]
S
[M]
SH
[M]
ArMs via covalent attachment
ArMs via supramolecular assembly
B
ncAAs for ArM design
M
N
N
BpyAla
M
O
N
HQAla
N
H
O
M = Cu, Zn
C
This work: in vivo incorporation of a ligand for noble-metal ArMs
pSHF
SH
H2N
COOH
OTS
LmrR
E. coli
Noble metal
e.g. Au(I)-precursor
SH
[Au]
S
NH2
H
N


A
LmrR_WT
LmrR_V15C
LmrR_V15pSHF
RuCp(MeCN)3PF6
[Ru(p-cymene)Cl2]2
[Ru(benzene)Cl2]2
[Rh(COD)Cl]2
Pd(OAc)2
AgNO3
[IrCp*Cl2]2
PtCl2
Pt(COD)Cl2
Pt(DMSO)2Cl2
Au(SMe2)Cl
NaAuCl4
≥5
Relative peak area metal adduct
0
B
SH
HS
Au(SMe2)Cl (2.0 equiv.)
50 mM NH4HCO3
pH 7.8, RT, 60 min
S[Au]
[Au]S
Intensity x 100000
[LmrR]+[Au]
15246.1 Da
[LmrR]+[O]
15064.2 Da
Mass (Da)
C
Abs
Wavelength (nm)
1.0 equiv. [Au]
0.5 equiv. [Au]
apo (dimer)
1.5 equiv. [Au]
2.0 equiv. [Au]
2.5 equiv. [Au]
3.0 equiv. [Au]
3.5 equiv. [Au]
4.0 equiv. [Au]

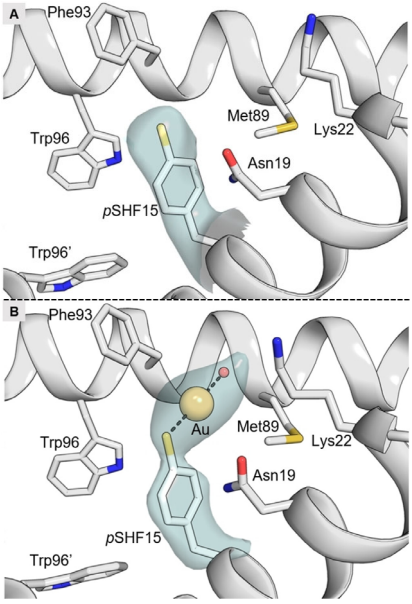
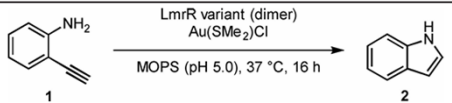
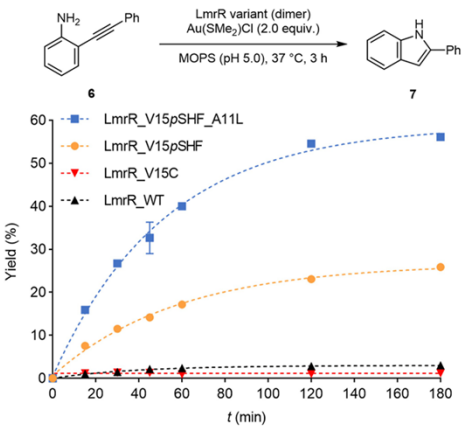


Table 1: Hydroamination reaction catalysed by different LmrR variants.[a]

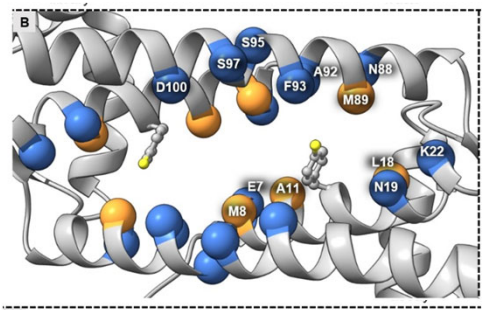


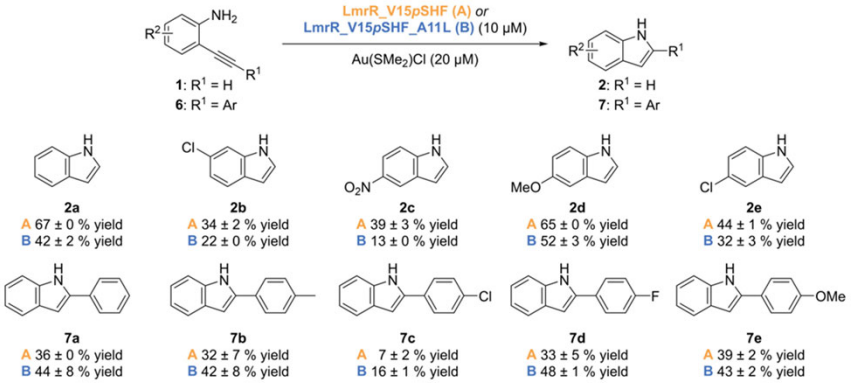
Entry	LmrR variant	Au equiv.	TON
1	LmrR_V15*p*SHF	2.0 equiv. (20 μM)	56 ± 12
2	LmrR_V15*p*SHF	1.0 equiv. (10 μM)	0 ± 0
3	LmrR_V15*p*SHF	–	0 ± 0
4	No protein	2.0 equiv. (20 μM)	4 ± 0
5	LmrR_WT	2.0 equiv. (20 μM)	21 ± 4
6	LmrR_V15C	2.0 equiv. (20 μM)	9 ± 1
7	LmrR_V15Y	2.0 equiv. (20 μM)	17 ± 3

[a] Reaction conditions: 1 mM substrate **1a**, 1 mol% ArM, 20 mM MOPS, 150 mM NaCl, pH 5.0, 2.6%v/v MeCN, 37 °C, 850 rpm, 16 h. Yields and conversions are obtained by GC-FID using mesitylene as internal standard. Results are obtained as an average of two experiments, errors are given as ± (standard deviation).



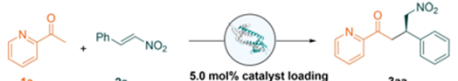
Scheme 3. Time course of gold ArMs catalysing the intramolecular hydroamination of substrate **6a**. Relative activity of LmrR variants, displayed by yield of **7a** over time. Reaction conditions: 200 μM substrate **6a**, 5 μM LmrR variant, 10 μM $Au(SMe_2)Cl$, 20 mM MOPS, 150 mM NaCl, pH 5.0, 2.6%v/v MeCN, 37 °C, 3 h. Results are obtained as an average of two experiments, and errors are given as ± (standard deviation).





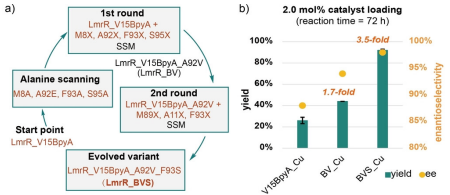
Scheme 4. Substrate scope of the hydroamination of various 2-ethynyl anilines with LmrR_V15*p*SHF–Au and LmrR_V15*p*SHF_A11L–Au. Reaction conditions: 1 mM substrate, 10 μM LmrR variant, 20 μM $Au(SMe_2)Cl$, 20 mM MOPS, 150 mM NaCl, pH 5.0, 2.6%v/v MeCN, 37 °C, 16 h. Yields are obtained by SFC using 2-phenylquinoline as internal standard. Results are obtained as an average of two experiments, and errors are given as ± (standard deviation).

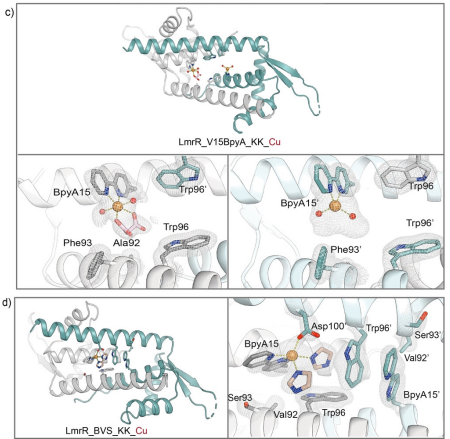
Table 1: Results of artificial metalloenzymes catalyzed the formation of **3aa** from **1a** and **2a** and control reactions.[a]

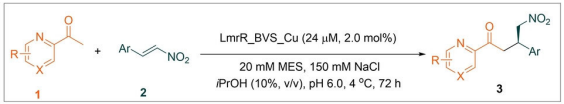


entry	catalyst	yield of **3aa**[b]	ee of **3aa**[b]
1	$Cu(NO_3)_2$	20 ± 2	0
2	$Cu(NO_3)_2$ + LmrR_WT	38 ± 1	47 ± 1
3[c]	$Cu(Phen)(NO_3)_2$ + LmrR_WT	7 ± 1	23 ± 1
4	$Cu(NO_3)_2$ + LmrR_V15BpyA	83 ± 1	93 ± 0
5	$Cu(NO_3)_2$ + LmrR_V15HQAla	30 ± 1	18 ± 0
6[d]	$Cu(NO_3)_2$ + LmrR_V15BpyA	89 ± 0	96 ± 0
7	LmrR_V15BpyA	0	/

[a] Reaction conditions: 2-acetyl pyridine **1a** (6.0 mM, 1.5 μmol, 5.0 equiv), trans *β*-nitro styrene **2a** (1.2 mM, 0.3 μmol, 1.0 equiv), copper precursor (60 μM, 5.0 mol%) loading with respect to LmrR_WT (60 μM, 5.0 mol%, concentration of dimer) or LmrR_V15BpyA (60 μM, 5.0 mol%, concentration of monomer), MOPS buffer (20 mM, pH 7.0, 150 mM NaCl), *i*PrOH (10% v/v), 4 °C, 72 h. [b] The yields and ee values of **3aa** were determined by SFC analysis with a chiral stationary phase using 2-phenyl quinoline as the internal standard. Yields and ee values are the average of at least two independent experiments, and all error values represent standard deviations. [c] $Cu(Phen)(NO_3)_2$: Lewis acidic Cu(II) complex of the ligand 1,10-phenanthroline. [d] MES buffer (20 mM, pH 6.0, NaCl 150 mM) was used instead of MOPS buffer.







3aa
LmrR_V15BpyA_Cu
26 ± 3% yield, 88 ± 1% ee
LmrR_BV_Cu
44 ± 1% yield, 94 ± 0% ee
LmrR_BVS_Cu
92 ± 1% yield, 98 ± 0 % ee

3ba[a]
97 ± 1% yield, 99 ± 0% ee

3ca[a]
30 ± 6% yield, 92 ± 1% ee

3da[a]
42 ± 3% yield, 84 ± 1% ee

3ea[b]
70 ± 4% yield, 1.8/1 dr
83 ± 1% ee (major)
45 ± 2% ee (minor)

3fa[c]
42 ± 1% yield, 85 ± 0% ee

3ab
79 ± 1% yield, 98 ± 1% ee

3ac[d]
91 ± 1% yield, 97 ± 0% ee

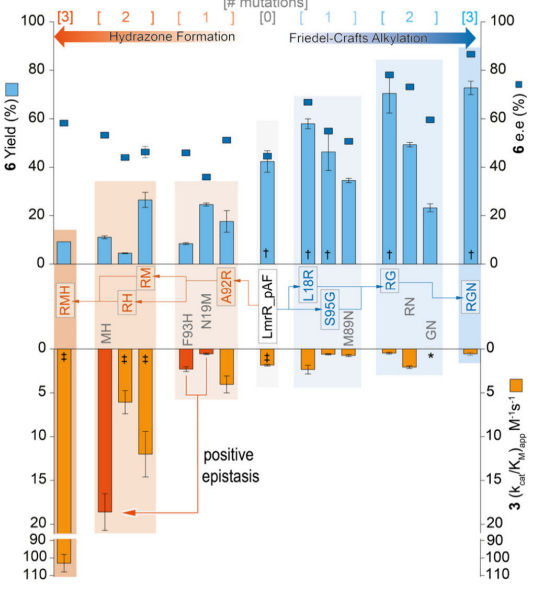
3ad[a, d]
62 ± 2% yield, 83 ± 1% ee

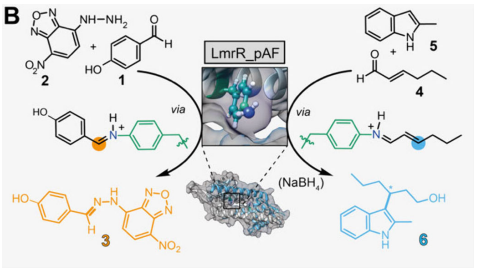
3ae, X = 4-Cl
90 ± 6% yield, 96 ± 0% ee
3af, X = 3-Cl
83 ± 1% yield, 98 ± 0% ee
3ag[c], X = 2-Cl
64 ± 2% yield, 74 ± 1% ee

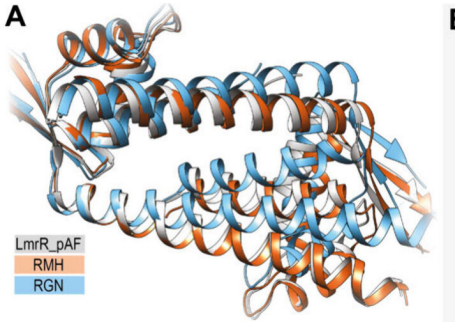
3ah
>99% yield, 98 ± 0% ee

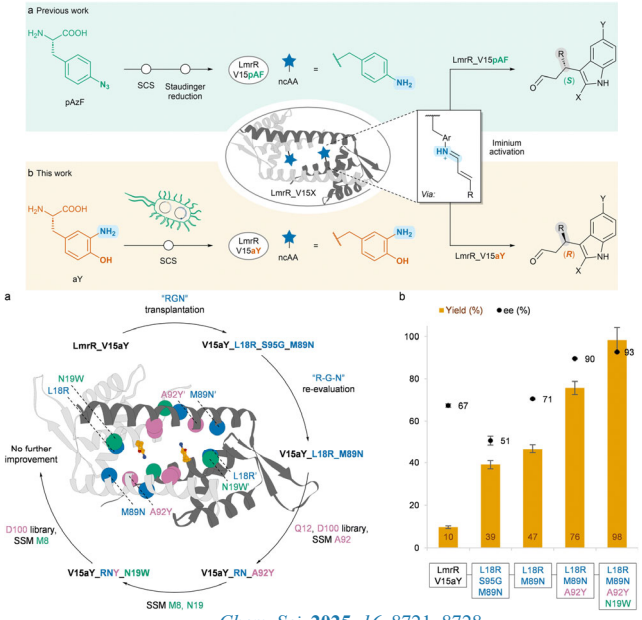
3ai, R = H
45 ± 1% yield, 95 ± 1% ee
3aj, R = Me
83 ± 1% yield, 98 ± 0% ee
3ak, R = Ph
52 ± 2% yield, 99 ± 0% ee

3al[d]
50 ± 1% yield, 96 ± 1% ee


[# mutations]
[3] [2] [1] [0] [1] [2] [3]
Hydrazone Formation
Friedel-Crafts Alkylation
6 Yield (%)
6 e.e (%)
3 ($k_{cat}/K_{M\,app}$, $M^{-1}s^{-1}$)
RMH
MH
RH
RM
F93H
N19M
A92R
LmrR_pAF
L18R
S95G
M89N
RG
RN
GN
RGN
positive epistasis


B
LmrR_pAF
via
via
(NaBH4)
2
1
5
4
3
6


A
LmrR_pAF
RMH
RGN



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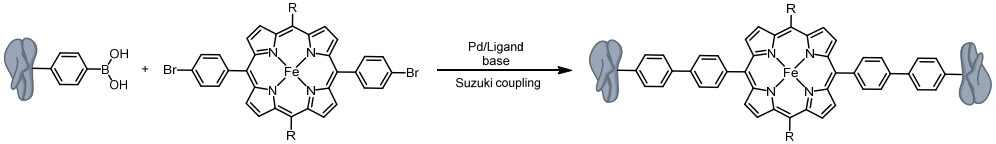
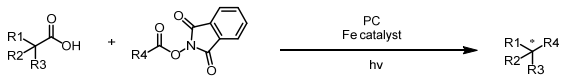
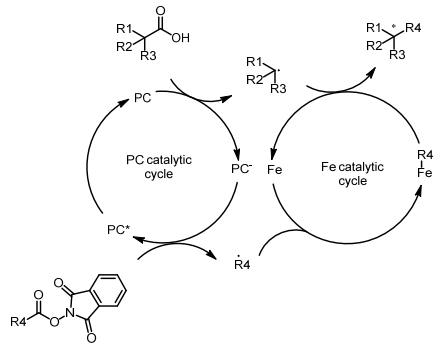

Pd/Ligand
base
Suzuki coupling
Fe


PC
Fe catalyst
hv


PC catalytic cycle
Fe catalytic cycle
PC
PC*
PC-
Fe

Thanks!