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An enantioselective artificial Suzukiase based on the biotin–streptavidin technology†

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Introduction of a biotinylated monophosphine palladium complex within streptavidin affords an enantioselective artificial Suzukiase. Site-directed mutagenesis allowed the optimization of the activity and the enantioselectivity of this artificial metalloenzyme. A variety of atropisomeric biaryls were produced in good yields and up to 90% ee. The hybrid catalyst described herein shows comparable TOF to the previous aqueous-asymmetric Suzuki catalysts, and excellent stability under the reaction conditions to realize higher TON through longer reaction time.

Introduction

The palladium-catalyzed Suzuki–Miyaura cross-coupling reaction (SMC hereafter) of organic halides with boronic acids is one of the most versatile methods for the synthesis of biaryls.^{1–3} Such structural motifs are present in numerous agrochemicals, natural products, pharmaceuticals, and polymers.^{4,5} For this purpose, the SMC has been widely studied.^{6–11} More recently, the SMC has found applications in the context of chemical biology. Indeed, both reactants and products can be regarded as bio-orthogonal.^{12–18} In this promising context however, high catalyst loadings are routinely required with reactions performed in a biological environment.^{12,13,15,17,19–27}

In stark contrast to the SMC, Nature relies on very different mechanisms to install (atropisomeric) C_{aryl}–C_{aryl} bonds.²⁸ Despite numerous reports on asymmetric SMC in organic media,^{6–8,29} only two reports describe enantioselective SMC in water.^{30,31} Uozumi reported on a heterogeneous SMC in water requiring a high catalyst loading (TON < 10, 94% ee at 80 °C).³⁰ More recently, Kündig *et al.* reported an asymmetric SMC in an water-organic solvent mixture (TON = 17, up to 80% ee) at room temperature.³¹ To complement these efforts, we speculated that, thanks to their well-defined second coordination sphere, artificial metalloenzymes (ArMs hereafter) may offer a propitious environment to engineer an asymmetric “Suzukiase”—that is, an enzyme that catalyzes the SMC. ArMs result from the incorporation of an abiotic cofactor within a macromolecule (protein or oligonucleotide).^{13,14,32–35} In this context, Ueno and coworkers anchored a palladium moiety within a ferritin container to yield

an artificial Suzukiase. The resulting artificial Suzukiase however did not outperform the free cofactor (no enantioselectivity, turnover frequency TOF: 3500 h^{−1}, no TON mentioned).¹³ Inspired by a seminal contribution by Whitesides in 1978,³² we report herein our effort to engineer an asymmetric artificial Suzukiase based on the biotin–streptavidin technology.

Results and discussion

With the aim of identifying suitable cross-coupling reaction conditions, we evaluated five different biotinylated catalyst precursors 1–5 in the presence of either avidin or streptavidin (Avi and Sav hereafter). For this purpose, the reaction of 1-iodonaphthalene **6c** with 2-methoxy-1-naphthaleneboronic acid **7c** was selected as a model system (Scheme 1). Initial experiments revealed that protonolysis of the boronic acid led to modest cross-coupling yields. Systematic variation of the base, pH, and organic co-solvent led to the identification of suitable reaction conditions: sodium hydroxide in DMSO : water (1 : 9) proved to be particularly effective in preventing protonolysis. However, competing deboronation required the use of excess boronic acid (1.5 equivalents *vs.* aryl halide) to achieve full conversion. Gel electrophoresis of the reaction mixture revealed that Sav remained largely tetrameric and active toward biotin binding even after an SMC performed at 50 °C (see ESI Fig. S3†).

Having identified suitable reaction conditions, we screened biotinylated phosphine- and NHC ligands 1–5 combined with WT (strept)avidin (Table 1). This screening led to the identification of complexes 1, 2, and 3 as the most promising catalysts in combination with WT Sav. Despite the structural similarity between avidin and streptavidin, the result obtained with avidin was inferior to those obtained with Sav. We thus focused on Sav for further optimization studies. Past experience with other ArMs based on the biotin–streptavidin technology suggest that mutations at positions S112 and K121 (which lie closest to the

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Scheme 1 Biotinylated cofactors 1–5 tested in the presence of (strept)avidin as artificial Suzukiase for the synthesis of enantioenriched 2-methoxy-1,1'-binaphthyl **8b**.

Table 1 Identification of the most suitable ligand for the synthesis of 2-methoxy-1,1'-binaphthyl **8b**^a

Entry	Complex	Protein	ee ^b [%]	TON
1	1	WT Sav	58 (<i>R</i>)	78
2	2	WT Sav	10 (<i>S</i>)	73
3	3	WT Sav	42 (<i>R</i>)	45
4	4	WT Sav	6 (<i>R</i>)	8
5	5	WT Sav	rac	<5
6	1	WT Avi	3 (<i>R</i>)	10

^a Reactions were carried out with 50 mM substrate in a total reaction volume 0.2 mL using 1 mol% complex 1–5 (see ESI for experimental details). ^b ee value determined by HPLC on a chiral stationary phase; absolute configuration assigned by comparison with literature data. WT = wild-type, Sav = streptavidin, Avi = avidin. All reactions were performed in duplicate: $\Delta ee = \pm 1\%$, $\Delta conv. = \pm 5\%$.

biotinylated metal cofactor) offer a versatile means to rapidly optimize the ArM's performance. Complexes 1–3 were thus screened with a small library of Sav mutants. The results of the chemogenetic optimization for the synthesis of 2-methoxy-1,1'-binaphthyl **8b** are displayed as a fingerprint in Fig. 1, and selected results are collected in Table 2.

From these data, the following features emerge:

(a) Compared to the free cofactor, higher conversions are observed with the artificial Suzukiase. This demonstrates that the Sav host protein exerts a beneficial influence on both the activity (*i.e.* protein accelerated catalysis)³⁶ and the selectivity.

(b) The electron-donating properties and bulkiness of the phosphine play a key role in determining the activity of the corresponding artificial Suzukiase. Accordingly, the (*t*-Bu)₂P-bearing catalysts outperform the (Ph)₂P-systems. Strikingly, both ligands 1 and 3 (Table 2, entries 10–13) show similar activity in case of S112M and S112A mutants.

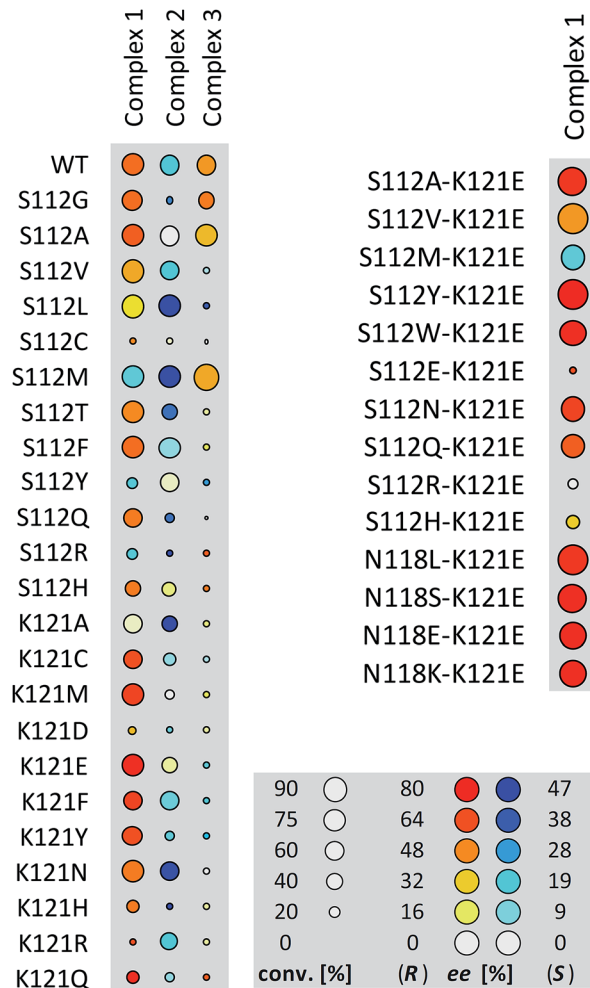


Fig. 1 Fingerprint display of the results of the chemogenetic optimization for the synthesis of 2-methoxy-1,1'-binaphthyl **8b**. The size of the circles is proportional to the conversion, and the color codes the enantiomeric excess. Numerical results are collected Table 2 and in Table S1 (ESI†).

(c) The enantioenriched nature of the cross-coupled product **8b** strongly supports the hypothesis that the SMC is indeed catalysed by a homogeneous protein-embedded Pd-cofactor rather than Pd-nanoparticles.^{12,25,26,37}

(d) Varying the spacer between the biotin anchor and the P(*t*-Bu)₂ from ethyl to propyl affords opposite enantiomers of biaryl **8b** for a given Sav mutant. The best ees in favour of (*S*)-**8b** were obtained with 2·Sav K121A and 2·Sav S112M (Table 2, entries 8 and 9). These findings illustrate the versatility of chemogenetic optimization strategy: upon varying the length of the spacer the Pd-moiety experiences a very different second coordination sphere environment which is reflected in the enantioselectivity.

Having identified 1·Sav K121E as a promising first generation Suzukiase (Table 2, entry 3), we screened double mutants bearing a glutamate at position 121 (Fig. 1 and Table 2, entries 14–21):

(a) The double mutant 1·Sav S112A–K121E (Table 2, entry 20) gives better ee and TON than the single mutant Sav S112A (Table 2, entry 13). Likewise, 1·Sav S112Y–K121E gave the



Table 2 Selected results for genetic optimization of the artificial Suzukiase for the synthesis of 2-methoxy-1,1'-binaphthyl **8b**

Entry	Temp [°C]	Complex	Protein	ee [%]	TON
1	RT	1	—	rac	20
2	RT	1	WT	58 (<i>R</i>)	78
3	RT	1	K121E	76 (<i>R</i>)	50
4	RT	1	K121D	34 (<i>R</i>)	7
5	RT	1	K121Q	74 (<i>R</i>)	20
6	RT	1	K121M	67 (<i>R</i>)	59
7	RT	1	K121F	67 (<i>R</i>)	38
8	RT	2	K121A	47 (<i>S</i>)	32
9	RT	2	S112M	44 (<i>S</i>)	53
10	RT	3	S112M	38 (<i>R</i>)	78
11	RT	3	S112A	36 (<i>R</i>)	52
12	RT	1	S112M	14 (<i>S</i>)	58
13	RT	1	S112A	60 (<i>R</i>)	58
14	RT	1	N118K–K121E	74 (<i>R</i>)	73
15	RT	1	N118S–K121E	76 (<i>R</i>)	79
16	RT	1	N118E–K121E	76 (<i>R</i>)	75
17	RT	1	N118L–K121E	72 (<i>R</i>)	86
18	RT	1	S112W–K121E	76 (<i>R</i>)	64
19	RT	1	S112N–K121E	69 (<i>R</i>)	61
20	RT	1	S112A–K121E	70 (<i>R</i>)	80
21	RT	1	S112Y–K121E	80 (<i>R</i>)	90
22	RT	1	S112Y–K121E	80 (<i>R</i>)	160 ^a
23	16	1	S112Y–K121E	84 (<i>R</i>)	50
24	4	1	S112Y–K121E	86 (<i>R</i>)	50 ^b
25	4	1	S112Y–K121E	90 (<i>R</i>)	50 ^c

^a 0.50 mol% catalyst loading, 0.25 mol% Sav (tetramer) loading. ^b 0.50 mol% catalyst loading, 0.25 mol% Sav (tetramer) loading, after 7 days.

^c Preparative scale (100 μmol). All reactions were performed in duplicate: Δee = ±1%, Δconv. = ±5%.

highest turnover with good ee (Table 2, entry 22). This finding highlights the non-additive nature of multiple mutations.^{38,39} Decreasing the temperature to 4 °C leads to an improvement in enantioselectivity (86% ee, Table 2, entry 24) albeit at the cost of a slower rate.

(b) Increasing the ratio of complex **1** vs. Sav tetramer (S112Y–K121E mutant) from one to four leads to a gradual erosion of enantioselectivity (from 80 to 69% ee, see Table S4, ESI[†]). This suggests that an empty biotin binding site adjacent to a complex **1** within Sav is favorable for selectivity.

(c) On a preparative scale, up to 90% ee (*R*)-**8b**, were obtained using 0.50 mol% catalyst loading and 0.25 mol% Sav (tetramer) loading at 4 °C (Table 2, entry 25).

(d) Upon decreasing the catalyst loading, a TON = 160 (TOF < 10 h⁻¹) is obtained with a constant ee (Table 2 entries 21–22). This TON compares favourably with the other aqueous asymmetric homogeneous SMC catalysts (TON = 10,³⁰ and 17 respectively³¹) compared to the achiral Suzukiase reported by Ueno¹³ however, the TOF is significantly lower (compare TOF 3500 h⁻¹ vs. <10 h⁻¹).

The artificial Suzukiase performed well on a range of hydrophobic substrates, leading to the following observations (Table 3, Fig. 2):

(a) The nature of the aryl halide does not influence the enantioselectivity but to lower conversions on going from iodide to bromide to chloride **6a–c** (Table 3, entries 6–8).



Fig. 2 Substrates tested for the asymmetric Suzuki–Miyaura cross-coupling reaction catalysed by **1**·S112Y–K121E.

(b) Substitution of the methoxy group by either a methyl and ethoxy group on the arylboronic acid (compare **7c** to **7b** and **7d**) leads lower enantioselectivities (Table 3, entries 7, 10 and 12). Depending on the nature of the *ortho*-substituent of the aryl moiety, the CIP priorities are inverted, yielding the opposite absolute configuration (*e.g.* compare **8b** and **8d**) for the same spatial arrangement. With the exception of the biphenyl-binaphthyl **8h**, all SMC products afford the same spatial arrangement using **1**·S112Y–K121E Sav. We speculate that upon binding to the palladium cofactor, the bulky biphenyl group **7h** forces the catalyst into a different second coordination sphere environment, leading to the opposite enantiomer (and spatial arrangement) of the coupled product.

(c) The system affords the highest enantioselectivity for binaphthyls or naphthyl-*o*-biphenyl products (albeit with the opposite chirality) in the presence of one smaller coupling partner, (*e.g.* **6h**), the enantioselectivity is significantly lower.

X-ray structure

To gain structural insight on the second coordination sphere around the Pd moiety, crystals of Sav S112Y–K121E were grown



extremely challenging to predict or rationalize why a given mutation leads to an improvement in catalytic performance. The chemogenetic optimization strategy allows to address this challenge with a limited effort: combining a small library of m mutants with a small library of n biotinylated ligands affords an $m \cdot n$ diversity matrix. Current efforts are aimed at performing catalytic asymmetric SMC with artificial Suzukiases *in vivo*.

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